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American Chemistry Council
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December 18, 2003

**201-14929**



By Mail
Mike Leavitt, Administrator
U.S. EPA
P.O. Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program – Test Plan Submission from HERTG
Registration Number

Dear Administrator Leavitt:

The American Chemistry Council Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG) submits for review and public comment its test plan report, as well as related robust summaries, for the single chemical, Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6), under the Environmental Protection Agency's High Production Volume (HPV) Chemical Challenge Program. The HERTG understands that there will be a 120-day review period for the test plan report and that all comments generated by or provided to EPA will be forwarded to the HERTG for consideration.

Thank you in advance for your attention to this matter. If you have any questions regarding the test plan report or the robust summaries, or HERTG's activities associated with the Challenge Program, please contact Sarah McLallen at 703-741-5607 (telephone), 703-741-6091 (telefax) or Sarah_McLallen@americanchemistry.com (e-mail).

Sincerely yours,

cc: HERTG members

201-14929B

# HIGH PRODUCTION VOLUME (HPV)

# CHALLENGE PROGRAM



## TEST PLAN

For

**Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)**

**Prepared by**
**The American Chemistry Council**
**Petroleum Additives Panel**
**Health, Environmental, and Regulatory Task Group**

**December 2003**

**LIST OF MEMBER COMPANIES IN THE**
**HEALTH, ENVIRONMENTAL AND REGULATORY TASK GROUP**

The Health, Environmental, and Regulatory Task Group (HERTG) of the American Chemistry Council Petroleum Additives Panel includes the following member companies:

Chevron Oronite Company, LLC

Crompton Corporation

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Groupe SNPE

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

Rhodia, Inc.

## 1.0 INTRODUCTION

In March 1999, the American Chemistry Council (formerly the Chemical Manufacturers Association) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address for certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address testing information for the following substance:

- Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)

This document indicates the findings of the data review process, and sets forth a proposed test plan.

In preparing this test plan the following steps were undertaken:

Step 1: A review of the literature and confidential company data was conducted on the physicochemcial properties, mammalian toxicity endpoints, and environmental fate and effects for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6) using its CAS number, CAS name, and synonyms. Searches included the following sources: MEDLINE, BIOSIS, CANCERLIT, CAPLUS, CHEMLIST, EMBASE, HSDB, RTECS, EMIC, and TOXLINE databases; the TSCATS database for relevant unpublished studies on these chemicals; and standard handbooks and databases (e.g., Sax, CRC Handbook on Chemicals, IUCLID, Merck Index, and other references) for physicochemical properties.

Step 2: The compiled data was evaluated for adequacy in accordance with the EPA guidance documentation.

## 2.0 GENERAL SUBSTANCE INFORMATION

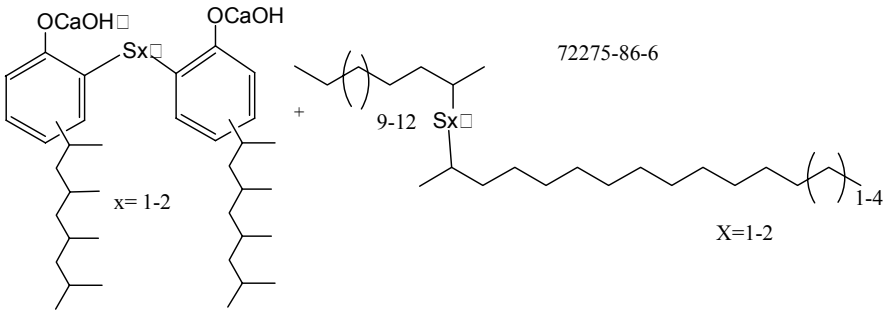
The substance that is the subject of this test plan is used as a petroleum additive in petroleum base stocks. The chemical name, CAS Registry Number, molecular weight and chemical structure for this substance are presented below.

Chemical Name: Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized

Chemical Abstract Service Registry Number: 72275-86-6

Molecular Weight Range: 454.88-699.13 gm/mol

Chemical Structure:




## 3.0 EXPOSURE INFORMATION

Manufacture

This material is prepared by sulfurizing tetrapropenyl phenol with excess elemental sulfur and neutralizing the sulfurized alkyl phenol with calcium hydroxide. In a second step in the same reactor after the reaction is cooled, a mixture of C15-C18 alpha-olefins is added. The olefins react with the remaining un-reacted sulfur producing a sulfurized olefin. The solvent in which these reactions occur is highly refined lubricating base oil, and the concentration of highly refined lubricant base oil in the commercial component is 19 wt.%.

This material is a physical mixture of CAS No. 67762-55-4, an alkyl sulfide that is a member of the Alkyl Sulfide category submission by the HERTG to the EPA HPV Chemical Challenge on March 28, 2000, and CAS No. 122384-85-4, an alkyl phenate sulfide that is a member of the Alkyl Phenate Sulfide category in preparation for submission by the HERTG to the ICCA HPV Chemical Challenge. Even though this material is made in one reaction, it remains a physical mixture of sulfurized calcium phenate and sulfurized alkyl sulfides. Both materials can be separated from the substance using HPLC indicating that there is no reaction between a sulfurized alkyl phenol and the additional olefin added in step two. This point justifies the use of data for the alkyl sulfide defined by CAS No. 67762-55-4 (or in some instances, data from a similar lower molecular weight alkyl sulfides defined by CAS No. 68511-50-2 and CAS No. 67124-09-8) and the alkyl phenate sulfide defined by CAS No. 122384-85-4 to augment the test data for CAS No. 72275-86-6 and supply data when data for CAS No. 72275-86-6 could not otherwise be located.

Use

This substance is used as a detergent and inhibitor in crankcase lubricants. It provides detergency to prevent deposit formation on engine parts such as on pistons and as an inhibitor to prevent bulk oil oxidation. The concentration of this substance ranges from 2.0 wt-% to 18.0 wt-% in additive packages. When these additive packages are blended into finished lubricants, the final concentration of this substance ranges from 0.5 wt-% to 3.0 wt-%.

## 4.0 PHYSICOCHEMICAL PROPERTIES

4.1 Summary of Available Data

The chemical structures for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized are shown in Table 2. These structures are essentially identical to an alkyl sulfide, Alkenes, C15-C18 alpha-, sulfurized (CAS No. 67762-55-4), and an alkyl phenate sulfide, Phenol, tetrapropenyl-, sulfurized, calcium salts (CAS No. 122384-85-4), and are similar to 1-Propene, 2-methyl-, sulfurized (CAS No. 68511-50-2), which is also an alkyl sulfide. The chemical structures of these substances are also shown in Table 2.

Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized are a liquid at ambient temperatures. The viscosity of this substance is 94 cSt at 100$^{o}$C, and the specific gravity is 1.00 at 15.6 $^{o}$C.

No published or unpublished data were located for the melting point, boiling point, vapor pressure, water solubility or octanol/water partition coefficient for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized. Data located for Phenol, tetrapropenyl-, sulfurized, calcium salts (CAS No. 122384-85-4) are also presented in Table 3.

4.2 Data Assessment and Test Plan for Physicochemical Properties

No adequate and reliable data were located for the boiling point and vapor pressure for alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized. However, Alkenes, C15-18 alpha, reaction product with sulfurized dodecyl phenol, calcium salt, sulfurized is liquid at ambient temperatures (thus melting point is not applicable). The octanol/water partition coefficient and water solubility of alkenes, C15-18 alpha, reaction product with sulfurized dodecyl phenol, calcium salt, sulfurized are indicative of the two structurally similar components (CAS Nos. 67762-55-4 and 122384-85-4). Therefore, testing is not proposed for water solubility or octanol/water partition coefficient. The other physicochemical properties will be measured as indicated in Table 1.

## 5.0 ENVIRONMENTAL FATE DATA

The environmental fate of a substance and its degradation by-products, including their partitioning among environmental compartments, are dependent on the physicochemical properties. The important environmental degradation pathways for lubricant additives are biodegradation, hydrolysis, and photodegradation.

### 5.1 Biodegradability

5.1.1 Summary of Available Data

Biodegradation, the measurement of the potential of a compound to be degraded by microorganisms, has been not been evaluated for Alkenes, C15-C18 alpha, reaction

products with sulfurized dodecyl phenol, calcium salt, sulfurized based on our inability to locate any studies in the published or unpublished scientific literature.

Biodegradation data exist for Phenol, tetrapropenyl-, sulfurized, calcium salts (CAS No. 122384-85-4). Although data could not be located for Alkenes, C15-C18 alpha-, sulfurized (CAS No. 67762-55-4), data are available for a structurally similar lower molecular weight alkyl sulfide, 1-Propene, 2-methyl-, sulfurized (CAS No. 68511-50-2). Data for both of these substances indicates that they are not readily biodegradable.

5.1.2. Data Assessment and Test Plan for Biodegradability

Based on the similarities in chemical structures and physicochemical properties for all of these substances, it is scientifically justifiable to extrapolate the data from the similar Alkyl sulfide and Alkyl phenate sulfide to Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6) and conclude that this substance also is not readily biodegradable. Therefore, additional biodegradation testing is not proposed.

## 5.2 Hydrolysis

5.2.1 Summary of Available Data

No published or unpublished hydrolysis data for members of the Alkenes, C15-18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized were located.

5.2.2 Data Assessment and Test Plan for Hydrolysis

Hydrolysis, the reaction in which a water molecule or hydroxide ion substitutes for another atom or group of atoms present in an organic molecule, has not been evaluated for Alkenes, C15-18 alpha, reaction products with sulfurized dodecyl phenol, calcium salts, sulfurized. However, an examination of the chemical structure suggests that Alkenes, C15-18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized, do not contain functional groups that are susceptible to hydrolytic degradative mechanisms.[1] Therefore, this fate process will not contribute to the degradative loss of chemical components in this category from the environment. Since Alkenes, C15-18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized, do not contain functional groups that are susceptible to hydrolytic degradative mechanisms, testing these substances for hydrolysis is not needed to adequately evaluate this endpoint. Therefore, no hydrolysis testing is proposed for this category**.**

## 5.3 Photodegradation

5.3.1 Summary of Available Data

[1] Lyman, W. J., W. F. Reehl, and D. H. Rosenblatt. 1982. Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co., New York, NY, USA.

No published or unpublished photodegradation studies for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized were located.

5.3.2 Data Assessment and Test Plan for Photodegradation

The Atmospheric Oxidation Potential (AOP) will be characterized for each of the separate components (CAS Nos. 67762-55-4 and 122384-85-4) of Alkenes, C15-18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized using the modeling program AOPWIN. This computer simulation is recommended in the Agency's recently released structure activity review (SAR) guidance for HPV chemicals.

### 5.4 Fugacity Modeling

5.4.1 Summary of Available Data

No published or unpublished fugacity-based multimedia fate modeling studies were located for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized.

5.4.2 Test Plan for Fugacity

The relative distribution of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized among environmental compartments will be evaluated using Level I Fugacity modeling. EPA states in the document, "Determining the Adequacy of Existing Data", that Level I fugacity modeling is acceptable to estimate transport/distribution values. The Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized environment.

Input data to run the Level I model will require an additional computer model to estimate physical/chemical properties from a structure if measured values are not available. The model used for this purpose will be EPIWIN, version 3.02[2], which was developed by the Syracuse Research Corporation. EPIWIN includes algorithms for estimating all physical and chemical properties needed for the Level 1 model.

## 6.0 ECOTOXICOLOGY DATA

### 6.1 Aquatic Toxicity

6.1.1 Summary of Available Data

- OECD Guideline 203 (*Fish, Acute Toxicity Test*): The 96-hour $LL_{50}$ of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized determined in sheepshead minnow is > 10,000 mg/L WAF. The 96-hour NOEL is 1000 mg/L WAF.

[2] Environmental Science Center- Syracuse Research Corporation- EPI for windows.

- The 96-hour $EL_{50}$ of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized determined in brown shrimp is 2600 mg/L WAF. The 96-hour NOEL is 100 mg/L WAF.

- Studies in daphnia (OECD Guideline 202, *Daphnia sp., Acute Immobilization Test and Reproduction Test*) or algae (OECD Guideline 201, *Alga, Growth Inhibition Test*) were not located in the published or unpublished scientific literature.

  Acute aquatic toxicity data in three species exist for Phenol, tetrapropenyl-, sulfurized, calcium salts (CAS No. 122384-85-4). Although data could not be located for Alkenes, C15-C18 alpha-, sulfurized (CAS No. 67762-55-4), data in three species exist for a structurally similar lower molecular weight alkyl sulfide, 1-Propene, 2-methyl-, sulfurized (CAS No. 68511-50-2). The data generally indicates that these two substances are of low concern for acute aquatic toxicity.

6.2.1 Data Assessment and Test Plan for Acute Aquatic Toxicity

The available acute aquatic toxicity data in fish and brown shrimp are adequate and reliable and are consistent with the data for the similar Alkyl sulfide and Alkyl phenate sulfide. Based on the similarities in chemical structure and physicochemical properties for all of these substances, it is scientifically justifiable to extrapolate the data for daphnia and algae from the similar Alkyl sulfide and Alkyl phenate sulfide to Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6) and conclude that this substance is a low concern for acute aquatic toxicity as well. Therefore, additional acute aquatic toxicity testing is not proposed.

## 7.0 MAMMALIAN TOXICOLOGY DATA

### 7.1 Acute Mammalian Toxicity

7.1.1 Summary of Available Data

The acute toxicity of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized has been evaluated via the oral and dermal route:

- OECD Guideline 401 *Acute Oral Toxicity* (similar to method FHSA 16 CFR1500.3): The acute oral $LD_{50}$ in the rat is > 5.0 g/kg indicating a low concern for acute toxicity.

- OECD Guideline 402 (*Acute Dermal Toxicity*): The acute dermal $LD_{50}$ in the rabbit is > 5.0 indicating a low concern for acute toxicity.

Acute toxicity data exists for the similar Alkyl sulfides and Alkyl phenate sulfide and are presented in Table 6. The results of these tests also indicate a low concern for acute toxicity.

7.1.2 Data Assessment and Test Plan for Acute Mammalian Toxicity

Adequate and reliable acute oral and dermal toxicity tests were performed for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized. Additional acute mammalian toxicity testing is not proposed.

## 7.2. Mutagenicity

7.2.1 Summary of Available Data

The genetic toxicity of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized has been evaluated for point mutations in test systems that evaluate base-pair substitution and frame shift mutations (Table 7):

- OECD Guideline 471 (*Bacterial Reverse Mutation Test*): A *Salmonella typhimurium* point mutation assay exists for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized. The data indicate that this substance is not mutagenic in this test system.

Studies of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized in test systems for chromosomal aberrations were not located.

Genetic toxicity studies for point mutations and chromosome aberrations exist for Phenol, tetrapropenyl-, sulfurized, calcium salts (CAS No. 122384-85-4). The data indicate that this substance is neither mutagenic nor clastogenic in these test systems.

Point mutation studies exist for structurally similar lower molecular weight alkyl sulfides, Alkenes, C15-C18 alpha-, sulfurized (CAS No. 67762-55-4) and 1-Propene, 2-methyl-, sulfurized (CAS No. 68511-50-2), and the data indicate that these substances are not mutagenic in this test system. In addition, a genetic toxicity study for chromosome aberrations exists for a C12-C16 analog of Alkenes, C15-C18 alpha-, sulfurized (CAS No. 67762-55-4), and the data indicate that this substance is not clastogenic in this test system.

7.2.2 Data Assessment and Test Plan for Mutagenicity Toxicity

An adequate and reliable *Salmonella typhimurium* point mutation assay is available for Alkenes, C15-C18 alpha-, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized. Additional adequate and reliable point mutation studies are negative for the similar Alkyl sulfide and Alkyl phenate sulfide. Based on the similarities in chemical structure and physicochemical properties for all of these substances, it is scientifically justifiable to extrapolate the data for chromosome aberrations from the similar Alkyl sulfides and Alkyl phenate sulfide to Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-

6) and conclude that this substance is a low concern for genetic toxicity as well. Therefore, additional genetic toxicity testing is not proposed.

## 7.3 Repeated-dose, Reproductive and Developmental Toxicity

### 7.3.1 Summary of Repeated-Dose Toxicity Data

The repeated dose toxicity of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized has been evaluated in a 28-day repeated-dose dermal toxicity study in rats (Table 8):

- OECD Guideline 410 (*Repeated Dose Dermal Toxicity: 21/28-day Study*): Minimal signs of systemic toxicity were observed in this study. The NOAEL is 100 mg/kg/day.

A repeated-dose toxicity study also exists for Phenol, tetrapropenyl-, sulfurized, calcium salts (CAS No. 122384-85-4). Although data could not be located for Alkenes, C15-C18 alpha-, sulfurized (CAS No. 67762-55-4), data exist for a structurally similar lower molecular weight alkyl sulfide, 2-propanol, 1-(tert-dodecylthio)- (CAS No. 67124-09-8). All reviewed studies indicate that these substances are also a low concern for repeated dose toxicity.

No published or unpublished reproductive or developmental toxicity tests for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized were located (Table 8). However, a reproductive toxicity screening toxicity test exists for phenol, tetrapropenyl-, sulfurized, calcium salts (CAS No. 122384-85-4). Although data could not be located for Alkenes, C15-C18 alpha-, sulfurized (CAS No. 67762-55-4), data exist for a structurally similar lower molecular weight alkyl sulfide, 2-propanol, 1-(tert-dodecylthio)- (CAS No. 67124-09-8). Both reproductive toxicity studies did not show any adverse effects on fertility or any other reproductive endpoints.

### 7.3.2 Data Assessment and Test Plan for Repeated-dose Toxicity

An adequate and reliable repeated-dose toxicity study exists for alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized. Although a reproductive toxicity study for this substance could not be located, adequate and reliable reproductive toxicity studies are negative for the similar Alkyl sulfide and Alkyl phenate sulfide. Thus, based on the similarities in chemical structure and physicochemical properties for all of these substances, it is scientifically justifiable to extrapolate the data for reproductive toxicity from the similar Alkyl sulfides and Alkyl phenate sulfide to Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6) and conclude that this substance is also a low concern for reproductive toxicity. Therefore, additional reproductive and developmental toxicity testing is not proposed.

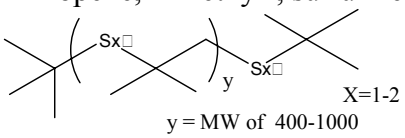
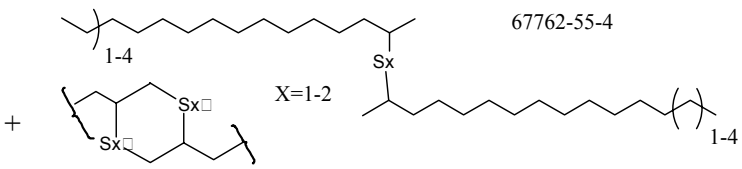
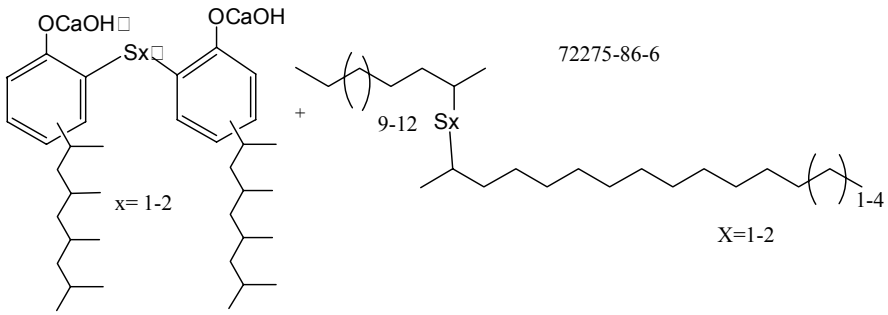
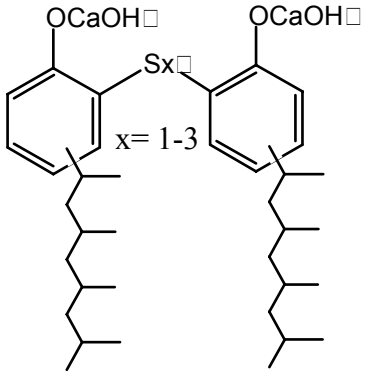
## SUMMARY

The following tables summarize the available data and proposed testing on alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized.

**Table 1**
**Summary Table of Available Data and Proposed Testing on**
**Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)**

| CAS No.: 7225-86-6 | Study Results | Testing Proposed |
|---|---|---|
| **Physical/Chemical Characteristics** | | |
| *Melting Point* | Liquid at ambient temperatures | No |
| *Boiling Point* | No Data Located | Yes |
| *Vapor Pressure* | No Data Located | Yes |
| *Water Solubility* | Indicative of two structurally similar components | No |
| *Partition Coefficient* | Indicative of two structurally similar components | No |
| **Environmental Fate** | | |
| *Biodegradation* | Not readily biodegradable | No |
| *Hydrolysis* | Not susceptible to hydrolytic degradative mechanisms | No |
| *Photodegradation* | No Data Located | Model with AOPWIN |
| *Fugacity* | No Data Located | Model with EPIWIN |
| **Ecotoxicity** | | |
| *Acute Toxicity to Fish* | 96 hr LC50: >10,000 mg/L WSF | No |
| *Acute Toxicity to Invertebrates* | 96 hr EL50 is 2600 mg/L WAF (brown shrimp)<br>96 hr NOEL is 100 mg/L WAF (brown shrimp) | No |
| *Acute Toxicity to Algae* | No Data Located<br>Bridging from similar substances | No |
| **Mammalian Toxicity** | | |
| *Acute Toxicity* | Oral LD50 > 5 g/kg (rat)<br>Dermal LD50 > 5 g/kg (rabbit) | No |
| *Repeated Dose Toxicity* | 30 wt% (300 mg/kg/day) in mineral oil<br>Changes in serum chemistry parameters<br>10 wt% (100 mg/kg/day) in mineral oil<br>No signs of systemic toxicity<br>3 wt% (30 mg/kg/day) in mineral oil<br>No signs of systemic toxicity | No |
| *Reproductive Toxicity*<br>*Developmental Toxicity* | No Data Located<br>Bridging from similar substances | No |
| **Genotoxicity** | | |
| *Gene Mutation* | Not mutagenic | No |
| *Chromosomal Aberration* | No Data Located<br>Bridging from similar substances | No |

**Table 2. Chemical Structures of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)**

| CAS Number | Chemical Structure |
|---|---|
| 68511-50-2<br>Alkyl Sulfide | 1-Propene, 2-methyl-, sulfurized<br>  <br><br>68511-50-2<br>y = MW of 400-1000 |
| 67762-55-4<br>Alkyl Sulfide | Alkenes, C15-C18 alpha-, sulfurized<br>  <br> |
| 72275-86-6<br>Reaction Mixture of Alkyl Sulfide and Alkyl Phenate Sulfides |  <br> |
| 122384-85-4<br>Alkyl Phenate Sulfide |  <br> |

## Table 3. Physicochemical Properties of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)

| CAS Number | Melting Point (°C) | Boiling Point (°C) | Vapor Pressure (Pa @ 25°C) | Water Solubility (mg/L) | Log Kow[5] |
|---|---|---|---|---|---|
| 68511-50-2 Alkyl Sulfide | 147.5-329.3[1] | 409.4-749.8[1] | $3.63 \times 10^{-16}$-$3.71 \times 10^{-5}$ [1] | $3.29 \times 10^{-18}$-$3.94 \times 10^{-4}$ | 7.99-15.23[1] |
| 67762-55-4 Alkyl Sulfide | 186.9-213.8[1] | 504.5-537.8[1] | $0.39\text{-}5.69 \times 10^{-8}$ [1] | $0.164\text{-}1.59 \times 10^{-10}$ [1] | 16.00-16.95[1] |
| 72275-86-6 | Liquid at ambient temperatures | Test | Test | No testing proposed Bridging | No Testing proposed Bridging |
| 122384-85-4 Alkyl Phenate Sulfide | 257[1] | 596[1] | $< 1.7 \times 10^{-4}$ [2] | 0.082[3] | > 6.6[3] |

[1] Modeling data, EPIWIN
[2] Based on partial pressure of the highly refined lubricant base oil [CONCAWE (1997) Lubricating Oil Basestocks. Product Dossier No. 97/108].
[3] Based on data for an overbased alkyl phenate sulfide (122384-87-6)

## Table 4. Evaluation of Environmental Fate Information for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)

| CAS Number | BIODEGRADABILITY Available Data & Proposed Testing | HYDROLYSIS Available Data & Proposed Testing | PHOTODEGRADATION Available Data & Proposed Modeling |
|---|---|---|---|
| 68511-50-2 Alkyl Sulfide | 0.3% degraded in 28 days | No data located | No data located |
| 67762-55-4 Alkyl Sulfide | No data located | No data located | No data located |
| 72275-86-6 | No testing needed Bridging | No testing proposed[1] | Model estimation[2] |
| 122384-85-4 Alkyl Phenate Sulfide | 1st Test: 7.8% biodegraded after 28 days 2nd Test: 13.4% biodegraded after 28 days | No data located | No data located |

[1] The structures that make up this substance do not contain functional groups that are subject to hydrolytic reactions. Therefore, these materials are expected to be stable in water and no testing is necessary.
[2] AOPWIN, a subroutine in EPIWIN, will be used to model potential indirect photodegradation rates for selected chemical structures that represent these substances.

## Table 5. Evaluation of Aquatic Toxicology of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)

| CAS Number | ACUTE TOXICITY TO FISH 96-hr $LL_{50}$ (mg/L)[1] | ACUTE TOXICITY TO INVERTEBRATES 48-hr $EL_{50}$ (mg/L)[1] | TOXICITY TO ALGAE 96-hr $EL_{50}$ (mg/L)[1] |
|---|---|---|---|
| | **Available Data & Proposed Testing** | **Available Data & Proposed Testing** | **Available Data & Proposed Testing** |
| 68511-50-2 Alkyl sulfide | > 10,000 ($WAF^2$,S)<br>> 1,000 ($WAF^2$,F) | > 1,000 ($WAF^3$, D) | > 100 ($WAF^3$, P, R)<br>34 ($WAF^3$, P, B) |
| 67762-55-4 Alkyl sulfide | No data located | No data located | No data located |
| 72275-86-6 | > 10,000 ($WAF^2$,S) | No testing proposed<br>Bridging | No testing proposed<br>Bridging |
| 122384-85-4 Alkyl phenate sulfide | > 1,000 ($WAF^2$,F)<br>> 1,000 ($WAF^2$,T) | > 1,000 ($WAF^3$, D)<br>>1,000 ($WAF^3$D)<br>96 hr EL50 is 2600 mg/L ($WAF^2$, BS)<br>96 hr NOEL is 100 mg/L ($WAF^2$, BS) | > 1,000 ($WAF^3$, P, B,R)<br>≥ 1,000 mg/L (algicidal)<br>≈ 200mg/L (algistatic) |

[1]Toxicity endpoints are expressed as median lethal loading rates ($LL_{50}$) for fish and median effective loading rates ($EL_{50}$) for *Daphnia* and algae. The $EL/LL_{50}$ is defined as the loading rate that adversely affects 50% of the test organisms exposed to it during a specific time. The greater the $EL/LL_{50}$ the lower the toxicity.
[2]WAF = Water accommodated fraction static renewal test
[3]WAF = Water accommodated fraction static non-renewal test
F = fathead minnow, *Pimephales promelas*
D = freshwater cladoceran, *Daphnia magna*
P = freshwater algae *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
T = rainbow trout, *Oncorhynchus mykiss;* formerly called *Salmo gairdneri*
S = sheepshead minnow, *Cyprinodon variegatus*
BS = brown shrimp (*crangon crangon)*
R = growth rate
B = biomass

## Table 6. Evaluation of Acute Toxicity of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)

| CAS Number | ACUTE ORAL TOXICITY[1] | ACUTE DERMAL TOXICITY[1] |
|---|---|---|
| | Available Data & Proposed Testing | Available Data & Proposed Testing |
| 68511-50-2<br>Alkyl sulfide | $LD_{50}$ > 5.0 g/kg (rat)<br>$LD_{50}$>5.0 g/kg (rat) | No data located |
| 67762-55-4<br>Alkyl sulfide | No data located | $LD_{50}$ > 2.0 g/kg (rabbit) |
| 72275-86-6 | $LD_{50}$ > 5.0 g/kg (rat) | $LD_{50}$ > 5.0 g/kg (rabbit) |
| 122384-85-4<br>Alkyl phenate sulfide | $LD_{50}$ > 5.0 g/kg (rat) | $LD_{50}$ > 2.0 g/kg (rat)<br>$LD_{50}$ > 15.0 g/kg (rabbit) |

[1]Toxicity endpoints are expressed as median lethal dose ($LD_{50}$) for acute oral and dermal toxicity. The $LD_{50}$ is defined as the dose that is lethal to 50% of the test organisms. The greater the $LD_{50}$, the lower the toxicity.

## Table 7. Evaluation of Genetic Toxicity of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)

| CAS Number | GENE MUTATION ASSAY | CHROMOSOMAL ABERRATION ASSAY |
|---|---|---|
| | Available Data & Proposed Testing | Available Data & Proposed Testing |
| 68511-50-2<br>Alkyl Sulfide | Bacterial Reverse Mutation Assay –<br>Not mutagenic | Mouse Micronucleus Assay –<br>Not clastogenic<br>Rat Micronucleus Assay –<br>Not clastogenic |
| 67762-55-4<br>Alkyl Sulfide | Bacterial Reverse Mutation Assay-<br>Not mutagenic | C12-C16 analog<br>Mouse Micronucleus Assay –<br>Not clastogenic |
| 72275-86-6 | Bacterial Reverse Mutation Assay –<br>Not mutagenic | No data located<br>No testing needed<br>Bridging |
| 122384-85-4<br>Alkyl<br>Phenate<br>Sulfide | Bacterial Reverse Mutation Assay –<br>Not mutagenic | Mouse Micronucleus Assay –<br>Not clastogenic |

## Table 8. Evaluation of Repeated-dose Mammalian Toxicity of Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6)

| CAS Number | REPEATED-DOSE TOXICITY | REPRODUCTIVE/DEVELOPMENTAL TOXICITY |
|---|---|---|
| | **Available Data & Proposed Testing** | **Available Data & Proposed Testing** |
| 67124-09-8 Alkyl Sulfide | **28-day repeated dose oral study in rats** NOAEL was not established in this study | One generation reproductive study in rats NOAEL = 50 mg/kg/day |
| 67762-55-4 Alkyl Sulfide | No data located | No data located |
| 72275-86-6 | **28-day repeated dose dermal study in rabbits** NOAEL (systemic toxicity) = 100 mg/kg/day | No data located<br>No testing needed<br>Bridging |
| 122384-85-4 Alkyl Phenate Sulfide | **4-week repeated dose oral study in rats** NOAEL = 300 mg/kg/day. | **Oral reproductive/developmental screening study in rats** NOAEL = 1000 mg/kg/day |

# 201-14929B

**Substance:** **Alkenes, C15-18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized**

**Summary prepared by:** **Petroleum Additives Panel**
**Health & Environmental Research Task Group**

## 1.0 Physico-chemical properties

**Summary 26-PhysChem-1**

| CAS # | N | Molecular Weight | Log $K_{ow}$ | Water Solubility (mg/L) | Vapor Pressure (Pa) | Log $K_{oc}$ | Log Bio-concen-tration Factor | Melting Point ($^{o}$C) | Boiling Point ($^{o}$C) | Atmospheric Oxidation | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | $OH^{-}$ Rate Constant ($cm^3$/molec-sec) | Half-life (hrs) |
| 68511-50-2 | y=3 | 410.8 | 7.99 | 3.94E-04 | 3.71E-05 | 6.12 | 3.45 | 147.52 | 409.48 | 35.69 | 3.60 |
| 68511-50-2 | y=8 | 851.6 | 15.23 | 3.29E-18 | 3.63E-16 | 11.98 | 0.5 | 329.32 | 749.88 | 90.67 | 1.42 |
| 67762-55-4 | y=2.5, x=1 | 497.0 | 16.00 | 1.64E-11 | 5.69E-08 | 9.50 | 0.5 | 186.92 | 504.54 | 77.22 | 1.66 |
| 67762-55-4 | y=2.5 x=2 | 529.0 | 16.95 | 1.59E-10 | 3.90E-09 | 9.77 | 0.5 | 213.83 | 537.80 | 300.52 | 22.63 |

**Summary 26-PhysChem-2**

| CAS # | Value | Method * | Year | Remarks |
|---|---|---|---|---|
| 122384-85-4 | Melting point: 257°C | calculated by Estimations Programs Interface for Windows; EPIWIN | 1999 | EPIWIN model validated using similar substances with known experimental values |
| 122384-85-4 | Boiling point: 596°C | calculated by Estimations Programs Interface for Windows; EPIWIN | 1999 | EPIWIN model validated using similar substances with known experimental values |
| 122384-85-4 | Vapor pressure: $< 1.7 \times 10^{-4}$ 2 | Based on partial pressure of the highly refined lubricating base oil | 1997 | CONCAWE, Lubricating Oil Basestocks, Product Dossier No. 97/108 |

**Robust Summary 26-PhysChem-3**

| | |
|---|---|
| ***Test Substance*** | |
| CAS # | CAS #122384-87-6 |
| Chemical Name | Phenol, tetrapropenyl-, sulfurized, carbonates, calcium salts, overbased |
| Remarks | Water solubility of 122384-85-4 is based upon this robust summary prepared for CAS # 122384-87-6. See Table 3 in the HERTG submitted test plan for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6) |
| ***Method/Guideline followed*** | Specialized method used due to very limited water solubility and difficulty in analysis. |
| GLP (Y/N) | N |
| Year (Study Performed) | 1996 |
| ***Test Conditions*** | Commercial material was placed in a dialysis bag and subjected to soxhlet extraction using hexane to remove diluent oil. Hexane was removed at 55C under a nitrogen stream. The material was loaded onto the generator column packing and water passed through the column at 0.7 ml/min. The effluent line from the generator column was connected to a C18 absorbent cartridge to trap any soluble material. Cartridge was removed from the system and any absorbed material removed by back-flushing with methanol, dichloromethane and methanol. The flushings were collected in a small glass vial. The solubility was determined by measuring the UV absorbance at 302 nm and comparing to the calibration curve generated by the standard. |
| ***Results*** | Water solubility = 0.082 mg/L. |
| Remarks | |
| ***Conclusions*** | The alkylphen(ol)ate sulfides have very low water solubility and the results of this experiment validates the QSAR modeled values. |
| ***Data Quality*** | (1) Reliable without restrictions |
| ***References*** | Rausina, G.A, W. R. Biggs, P. M. Stonebraker and E. A. Crecelius. Using Semipermeable Membrane Devices (SPMDs) to estimate bioconcentration potential of Petroleum Additives. Tribology – Solving Friction and Wear Problems, 10th International Colloquium Tribology, Stuttgart/Qstfildern, Germany. January 9-11, 1996. |
| ***Other*** | Dated: 2-22-03 |
| | |

**Robust Summary 26-PhysChem-4**

| | |
|---|---|
| ***Test Substance*** | |
| CAS # | CAS #122384-87-6 |
| Chemical Name | Phenol, tetrapropenyl-, sulfurized, carbonates, calcium salts, overbased |
| Remarks | Log Kow of 122384-85-4 is based upon this robust summary prepared for CAS # 122384-87-6. See Table 3 in the HERTG test plan for Alkenes, C15-C18 alpha, reaction products with sulfurized dodecyl phenol, calcium salt, sulfurized (CAS No. 72275-86-6) |
| ***Method/Guideline followed*** | Log Kow determined by reverse phase High Performance Liquid Chromatography (HPLC) |
| GLP (Y/N) | N |
| Year (Study Performed) | 1996 |
| ***Test Conditions*** | The Octanol/Water Partition Coefficient (OWPC) was run using reverse phase HPLC octadecyl bonded-phase chromatography column (Zorbax ODS, 25cmX 0.46 cm I.D.) and the following method (Chromatographia, V. 27, 118-122 (1989). The Calcium phenate sulfide was dissolved in acetonitrile and the same solvent was used as the mobile phase. Several polycyclic aromatic hydrocarbons (PAH) with known OWPCs were used as reference. The retention times of the PAHs were compared to that of the calcium phenate sulfide. Each PAH has a very distinct UV adsorption spectrum (using a 2-dimensional UV detector) so that the mixture of PAHs and calcium phenate sulfide could be run together and the OWPC for each component determined. |
| ***Results*** | Measured log Kow = 6.6; Elution Time = 2.4 |
| Remarks | |
| ***Conclusions*** | |
| ***Data Quality*** | (1) Reliable without restrictions |
| ***References*** | Rausina, G.A, W. R. Biggs, P. M. Stonebraker and E. A. Crecelius. Using Semipermeable Membrane Devices (SPMDs) to estimate bioconcentration potential of Petroleum Additives. Tribology – Solving Friction and Wear Problems, 10th International Colloquium Tribology, Stuttgart/Qstfildern, Germany. January 9-11, 1996. |
| ***Other*** | Dated: 6-2-03 |
| | |

## 2.0 Environmental Fate and Pathways

## 2.1 BIODEGRADABILITY

**Robust Summary 26-Biodeg-1**

| | |
|---|---|
| ***Test Substance*** | |
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | OECD 301B, Ready Biodegradability, Modified Sturm Test |
| Test Type (aerobic/anaerobic) | Aerobic |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1996 |
| Contact time (units) | 28 days |
| Inoculum | Domestic sewage sludge plus soil |
| Remarks for test conditions | Inoculum: Sludge from domestic WWTP used at 30 mg dry solids/L; soil from forest area used at 0.1 g/L<br><br>Conc of test chemical: Test chemical added directly to test vessels at 13.3 mg C/L (28.6 mg/L CAS# 68511-50-2). No preacclimation was used.<br><br>Temp of incubation: 23 – 24 °C<br>Dosing procedure: Neat test chemical added by micropipettor to culture medium in vessels immediately prior to addition of sewage and soil inocula<br><br>Sampling: Days 1, 3, 6, 10, 14, 21, 29 (after acidification on d 28)<br><br>Controls: Yes (blank and positive controls used per guideline); toxicity control not used. Positive Control was Benzoic acid (Na salt) at 20 mg C/L<br><br>Analytical method: Titration of residual Ba(OH)2 in trapping solution, using HCl<br><br>Method of calculating measured concentrations: N/A; CO2 evolution and % biodegradation were calculated using the average of duplicate blank-corrected titration volumes at each titration interval<br><br>Other:<br>• The % biodegradation value reported is slightly inflated by the use of zero titration volume rather than negative volume when corrected for blanks; however, comparison of titration volumes for the test chemical and blank show them to be very similar, so inhibition of inoculum is not suspected. |

| | |
|---|---|
| ***Results*** | Not Readily Biodegradable |
| Degradation % after time | 0.3% in 28 days |
| Kinetic (for sample, positive and negative controls) | $t_{1/2}$ for Positive Control was <10 d |
| Breakdown Products (Y/N) If yes describe breakdown products | N |
| ***Conclusions*** | Not Readily Biodegradable; biodegradation was essentially zero |
| ***Data Quality*** | Reliable without restrictions |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12/29/99 |

**Robust Summary 26-Biodeg-2**

| | |
|---|---|
| Test Substance | |
| CAS # | 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | Testing was performed a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenate sulfide and 40% highly refined lubricant base oil.<br><br>This substance is referred to as dodecyl derivative in the HERTG's Test Plan for Alkyl Phenate Sulfide Category. |
| Method | |
| Method/Guideline followed | Official Journal of the European Communities $CO_2$ Evolution Test (Method C. 4-C). |
| Test Type (aerobic/anaerobic) | Aerobic |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1996 |
| Contact time (units) | 28 days. |
| Inoculum | Activated sludge from domestic wastewater treatment plant. |
| Remarks for test conditions | Inoculum: The supernatant from the homogenized, settled activated sludge was used as inoculum. The supernatant was added to the test flasks at 1% v/v (20 mL added per flask), at an activity of $2.1 \times 10^5$ colony forming units per mL. Diluent in flasks was modified BOD water.<br><br>Concentration of test chemical: Approximately 0.27 g test material was added to each flask, giving 10 mg C/L in the test flasks.<br><br>Temp of incubation: 22.5 $\pm$ 1ºC.<br><br>Dosing procedure: No organic solvents were used to facilitate dissolution of the test material. Test material addition was performed by placing the measured amount of the test material onto a glass slide, then placing the slide into the test vessel.<br><br>Test Setup: Total volume of liquid in flasks was 2 L. Flasks were agitated on a shaker table during the 28-day test. Flasks were aerated with $CO_2$ free air, and generated $CO_2$ was collected in $BaCO_3$ as the air left the flasks.<br><br>Sampling frequency: Carbon dioxide traps were removed from the sampling train "periodically" and titrated to determine the amount of $CO_2$ trapped. At day 28, the test was terminated by the acidification of the test flasks to release dissolved $CO_2$.<br><br>Controls: Blank, positive and toxicity controls were included; abiotic controls were not. Sodium benzoate was used as the reference substance in the positive and toxicity controls. |

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| | Analytical method: $Ba(OH)_2$ ("trap") solutions were used downstream of the test flasks to trap generated $CO_2$ as $BaCO_3$. The used trap solutions were titrated to a phenolphthalein endpoint to determine the amount of $CO_2$ trapped.<br><br>Method of calculating measured concentrations: N/A<br><br>Other: Because the test was for ready biodegradability, the inoculum was not pre-exposed to the test substance before test initiation. |
| Results | |
| Test Substance Degradation, % after time | 7.8% after 28 days |
| Kinetic (for sample, positive and negative controls) | Reference (sodium benzoate): 88.1% (0.45 day lag period)<br>Test substance: 7.8% (28d)<br>Toxicity Control: 43.2% (28d; 0.85 day lag), approximately equivalent to 79.4% biodegradation for reference substance and 7.0% for test substance, so no measurable toxicity. |
| Breakdown Products (Y/N) If yes describe breakdown products | N |
| Remarks | |
| Conclusions | Test substance degraded 7.8% in 28 days. The reference substance, sodium benzoate, degraded 88.1% in the same test period and the toxicity test showed the test substance to be non-inhibitory, so the test was valid. |
| Data Quality | (1) Reliable without restrictions. |
| References | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| Other | Updated: 9-25-00 |

**Robust Summary 26-Biodeg-3**

| | |
|---|---|
| Test Substance | |
| CAS # | 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts |
| Remarks | Testing was performed a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenate sulfide and 40% highly refined lubricant base oil.<br><br>This substance is referred to as dodecyl derivative in HERTG's Test Plan for Alkyl Phenate Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of "Alkyl Phenate Sulfide Category" in HERTG's Test Plan for Alkyl Phenate Sulfide Category. |
| Method | |
| Method/Guideline followed | OECD 301B |
| Test Type (aerobic/anaerobic) | Aerobic |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1998 |
| Contact time (units) | 28 days. |
| Inoculum | Activated sludge from domestic wastewater treatment plant. |
| Remarks for test conditions | Inoculum: The supernatant from the homogenized activated sludge was used as inoculum. The sludge was homogenized in a blender at medium speed for approximately 2 minutes and allowed to settle for approximately 30 minutes. The supernatant was used the same day that it was prepared. The average measured total suspended solids (TSS) concentration of the inoculum was 78.6 mg/L. The standard plat count showed 1.37E+06 CFU/mL.<br><br>Concentration of test chemical: An amount of test substance necessary to deliver 10 mg Carbon/L was added to the treatment group by direct weight addition.<br><br>Temp of incubation: 20 ± 3ºC.<br><br>Dosing procedure: No organic solvents were used to facilitate the dispersion of the test material. Similar procedure was employed with the reference substance.<br><br>Test Setup: The final volume within all chambers was 3000 mL. CO2 free air was bubbled through each of the test chambers at a rate of 50 to 100 mL per minute. The contents of the test chambers were mixed with the aid of magnetic stir bars and stir plates.<br><br>Sampling frequency: The CO2 traps were removed for analysis on days 1,4, 8, 11, 14, 19, 21, 25, and 29. The CO2 trap nearest the chamber was removed and analyzed for inorganic carbon. On day 28, the test was terminated by the acidification of the test flasks to release dissolved CO2. |

| | |
|---|---|
| | Controls: Yes (blank and positive controls per guideline). Abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control.<br><br>Analytical method: The CO2 produced within the test chamber was trapped as K2CO3 in the KOH solution and the amount of inorganic carbon in the trapping solution was measured at regular intervals during the study, using a Shimadzu TOC-5000 carbon analyzer.<br><br>Method of calculating measured concentrations: N/A<br><br>Other: The inoculum was not pre-adapted to the test material. |
| Results | |
| Degradation % after time | 13.4% after 28 days |
| Kinetic (for sample, positive and negative controls) | Reference (sodium benzoate) – 99.3% (28d). An average percent biodegradation of >60% was achieved within 7 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14.<br>Test substance – 13.4% (28d) |
| Breakdown Products (Y/N) If yes describe breakdown products | N |
| Remarks | |
| Conclusions | Test material degraded 13.4% in 28 days. The reference substance, sodium benzoate, reached a level of 88.8% in the same test period. |
| Data Quality | (1) Reliable without restrictions. |
| References | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| Other | Updated: 9-22-2000 |

## 3.0 AQUATIC ORGANISMS

### 3.1 Acute and Prolonged Toxicity to Fish

### Robust Summary 26-Fish-1

| ***Test Substance*** | |
|---|---|
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is referred to as methyl propene derivative in the HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984). |
| Test Type | WSF static renewal test; a one level screening test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1986 |
| Species/Strain | Sheepshead minnow (*Cyprinodon variegatus*) |
| Analytical Monitoring | Total organic carbon (TOC) measurements of each freshly prepared test solution and control and after 24-h on test just before daily renewal with fresh test solution. |
| Exposure Period (unit) | 96 hours |
| Statistical methods | Statistical analysis of survival data not warranted. |
| Remarks field for test conditions (fill as applicable) | Test Organisms: source – a commercial supplier in New Hampshire, age – 24 to 29 days old, total length – 20.3 mm average (range 13 to 30 mm; n =18), wet weight – 0.16 g average (range 0.03 to 0.039 g; n = 18). Loading - 0.32 g biomass/L, Pretreatment – none, fish held for a minimum of 20 days before testing. No feeding during the test.<br><br>Test System: Individual WSFs were prepared for each daily renewal of the 10,000 mg/L test level. A measured weight of test material was added to a measured volume of dilution water (15-L) in a glass vessel and stirred for 16 to 24 hours. Stirring accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 2 hours before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end approximately midway between bottom and surface. The siphoned water phase, designated water soluble fraction (WSF), was used for the aquatic toxicity test. About 90% of the test solution in each test vessel was renewed daily after 24, 48, and 72 hours. Two 5-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels were loosely covered to reduce entry of dust, etc. |

| | |
|---|---|
| | Dilution Water: Natural seawater collected from Cape Cod Canal, Bourne, Massachusetts, which derives water from Buzzards Bay or Massachusetts Bay. The water was filtered through 0.5-micron polypropylene core filter and activated carbon, then stored for 1 to 4 days prior to use while being constantly aerated. During storage the water had a salinity of 32 to 33 ppt and pH of 7.7 to 7.8. During the test: dissolved oxygen – 5.6 mg/L to above 100% saturation (7.5 mg/L), pH – 6.9 to 8.0, salinity – 32, temperature – 20 to 22 C. Mean measured TOC levels in the control and 1,000 mg/L WSF test level were 2.7 mg/L (range 1.2 to 6.0) and 4.5 mg/L (range 3.0 to 5.6), respectively<br><br>Test Levels: Control & 10,000 mg/L WSF loading rate.<br><br>Test Findings: No mortality or signs of toxicity were noted in the 10,000 WSF test level and the control throughout the entire test.<br><br>Calculation of $LL_{50}$ s: Statistical analysis of survival data not warranted.<br><br>Test Substance: No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test.<br><br>Reference Substance: Sodium lauryl sulfate (SLS). The 96-h $LC_{50}$ was 1.2 mg/L. No information provided on method of calculation. |
| ***Results*** | Nominal concentrations: 96-h $LL_{50}$ >10,000 mg/L. 96-h $LL_0$ = 10,000 mg/L (no mortality or toxic signs noted). Mean measured TOC in the 10,000 mg/L WSF test level was 4.5 mg/L compared to 2.7 mg/L in the control. |
| Remarks | Measured concentration: n/a<br><br>Unit: mg/L<br><br>LC50, LC0, LL50 or LL0 at 48, 72, 96 hours: $LL_{50}$ and $LL_0$ reported as $LC_{50}$ and NOEC, respectively, although test results were based on WSF loading rate.<br><br>Statistical results: Statistical analysis of survival data not warranted.<br><br>Other:<br>• |

| | |
|---|---|
| ***Conclusions*** | No mortality or signs of toxicity were noted in the 10,000 WSF test level and the control throughout the entire test. |
| ***Data Quality*** | Reliable without restrictions |
| ***References*** | Chemical Manufacturers Association, HERTG<br><br>Nicholson, R.B. (1986) Acute toxicity of CMA Test Material Code 525 to Sheepshead Minnow, *Cyprinodon variegatus.* Springborn Bionomics Study #10823-0186-6100-500-525, Report #BW-86-04-2004. |
| ***Other*** | Updated: 12-21-99 |
| | |

**Robust Summary 26-Fish-2**

| ***Test Substance*** | |
|---|---|
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is referred to as methyl propene derivative in the HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984). |
| Test Type | WAF static renewal test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1993 |
| Species/Strain | Fathead minnow (*Pimephales promelas)* |
| Analytical Monitoring | Total organic carbon (TOC) measurements of initial test solutions and control (0-hour) and after one day on test (24-h) before daily renewal of fresh test solution. |
| Exposure Period (unit) | 96 hours |
| Statistical methods | Statistical analysis of survival data not warranted. |
| Remarks field for test conditions (fill as applicable) | Test Organisms: source – Aquatic Research Organisms, Hampton, New Hampshire, age – juvenile, total length – 25 mm average (longest fish not more than twice the shortest fish), wet weight – 0.1 g average (no range reported). Loading - <0.5 g biomass/L, Pretreatment – none, fish held for a minimum of 14 days before testing. No feeding during the test.<br><br>Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (30-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used in the aquatic toxicity test. About 80% of the solution in each test level was renewed daily after 24, 48, and 72 hours. Two 15-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels loosely covered to reduce entry of dust, etc.<br><br>Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. The water was characterized as moderately hard water. Alkalinity not reported. Dissolved oxygen – 7.1 to 8.7 mg/L, pH – 7.8 to 8.5, conductivity – 570 to 650 umhos/cm, |

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| | temperature – 21.4 to 22.8 C. TOC levels were between 2 to 3 mg/L in the control, 3 mg/L at 100 mg/L test level, between 3 to 4 mg/L at 300 mg/L test level and 5 mg/L at 1,000 mg/L test concentration level.<br><br>Test Levels: Control, 100, 300 & 1,000 mg/L WAF loading rates.<br><br>Test Findings: No mortality was observed in all treatments and the control throughout the entire test and no signs of toxicity were noted in all treatments throughout 72 hours. At 96 hours, all 20 fish in the 1,000 mg/L test level were lethargic and exhibited erratic swimming, but no signs of toxicity were observed in the lower test levels and control.<br><br>Calculation of $LL_{50}$ s: Statistical analysis of survival data not warranted.<br><br>Test Substance: No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test.<br><br>Reference Substance: No |
| ***Results*** | Nominal concentrations: 96-h $LL_{50}$ >1,000 mg/L. 96-h $LL_0$ = 300 mg/L. No mortality at 1,000 mg/L but at 96 hours all fish were lethargic and exhibited erratic swimming. TOC measurements at 1,000 mg/L were 5 mg/L compared to 2 to 3 mg/L in the control. |
| Remarks | Measured concentration: n/a<br><br>Unit: mg/L<br><br>LC50, LC0, LL50 or LL0 at 48, 72, 96 hours: $LL_{50}$ and $LL_0$ reported as $LC_{50}$ and NOEC, respectively, although test results were based on WAF loading rates.<br><br>Statistical results: Statistical analysis of survival data not warranted.<br><br>Other:<br>• The TOC in dilution water at the beginning and end of the test was greater than 2 mg/L rather than <2 mg/L. It could not be verified that water samples were passed through a 0.45 micron filter prior to TOC analysis, that the test vessels were covered, or that the continuous temperature measurement was made in a control vessel during the study. But, these deviations did not compromise the study. |

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| ***Conclusions*** | No mortality was observed in all treatments and the control throughout the entire test and no signs of toxicity were noted in all treatments throughout 72 hours. At 96 hours, all 20 fish in the 1,000 mg/L test level were lethargic and exhibited erratic swimming, but no signs of toxicity were observed in the lower test levels and control. |
| ***Data Quality*** | Reliable without restrictions |
| ***References*** | Chemical Manufacturers Association, HERTG<br><br>Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 613 to The Fathead Minnow, *Pimephales promelas*. T.R. Wilbury Study #9176-CMA/ESI-613. |
| ***Other*** | Updated: 12-21-99 |
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**Robust Summary 26-Fish-3**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 72275-86-6 |
| Chemical Name | Alkenes, C15-18 alpha, reaction products with sulfurized tetrapropenyl phenol, calcium salt, sulfurized |
| Remarks | Test material purity not provided. |
| ***Method*** | |
| Method/Guideline followed | OECD 203 |
| Test Type | 96 Hour Acute Toxicity to Fish (Water soluble fraction, static renewal) |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1989 |
| Species/Strain | Sheepshead Minnows (Cyprinodon variegatus) |
| Fish Number | 10/concentration/replicate (20/concentration total) |
| Fish Size | Average length 3.7-4.3 cm; Average weight 1.37-1.98 g (two batches used during the study) |
| Analytical Monitoring | No |
| Nominal Test Substance Concentration Levels | 0 (control), 100, 500, 1,000 and 10,000 mg/l water soluble fraction tested in duplicate |
| Test Concentration Preparation | Test solutions (water soluble fractions) were prepared separately for each replicate test concentration by adding an appropriate aliquot (by weight) of test material in diluent water and stirring overnight. The aqueous phase was then withdrawn for testing. A separate extraction was prepared for each exposure level. |
| Exposure Period | 96 hours |
| Exposure Conditions | Static renewal (daily) test conditions. |
| Vehicle | None |
| Statistical Analysis | None required based on the results. |
| Dose Range finding Study | No |
| Test Chambers | Glass aquaria containing 20-liters of test solution and covered with aluminum foil to reduce volatilization |
| Diluent Water | Synthetic sea water (Synthetica) aerated for at least 20 hours prior to use. |
| Water chemistry during exposures | Temperature: $22^{O}C$<br>Dissolved oxygen (mg $O_2$/L): 7.9-8.0<br>PH: 8.3-8.4 |
| Photoperiod | 16 hours of light, 8 hours of dark |
| Positive Control | None |
| Remarks field for test conditions | All fish were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 3, 6, 24, 48, 72, and 96 hours after initiation of test material exposure was recorded. Measurements of pH, dissolved oxygen and temperature were recorded daily in each test chamber. Chambers were aerated to give $\geq$80% air saturation. Chamber loading was at < 1 gram of body weight/L. Fish were fed commercial flake fish food during acclimatization. Feeding was discontinued 24 hours prior to study initiation. Once daily, surviving fish were temporarily removed from the exposure chambers by gentle netting. The fish were moved to a small volume of test medium, while each chamber was thoroughly cleaned. Each chamber was refilled with fresh test solution and the fish were returned to the chambers. |

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| ***Results*** | All fish survived the 96-hour duration of the study at all concentrations. At 10,000 mg/L, 1 of 10 fish in the first replicate and 1 of 10 fish from the second replicate were found alive, lying on the bottom of the chamber, at 48 hours and 2 of 9 fish from the second replicate were found alive, lying on the bottom of the chamber at 72 hours (4 of 20 fish total). One fish was moribund at this exposure level at 96 hours.<br><br>The 24, 48, 72 and 96-hour LC50s were each greater than 10,000 mg/L (nominal concentration-water soluble fraction). The 24-hour no observed effect level was 10,000 mg/L. The 48, 72 and 96 hour no observed effect levels were 1,000 mg/L. |
| ***Conclusions*** | Under the conditions of this study the 24, 48, 72 and 96-hour LC50s were each greater than 10,000 mg/L (nominal concentration-water soluble fraction). The 24-hour no observed effect level was 10,000 mg/L. The 48, 72 and 96 hour no observed effect levels were 1,000 mg/L. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of exposure concentration. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 3/28/2003 |
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**Robust Summary 26-Fish -4**

| ***Test Substance*** | |
|---|---|
| CAS # | 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, , calcium salts |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenol sulfide and 40% highly refined lubricant base oil. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline 40 CFR Part 797 (1993), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1992). |
| Test Type | Static renewal test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1997 |
| Species/Strain | Rainbow trout (*Oncorhynchus mykiss*) |
| Analytical Monitoring | Total organic carbon (TOC) measurements were performed on initial (0-h) and 24 hour (before renewal) control and 1000 mg/L loading test solutions . Water samples were not filtered prior to TOC analysis using EPA Method 415.1 |
| Exposure Period (unit) | 96 hours |
| Statistical methods | Statistical analysis of survival data not warranted because there was at least 90% survival in both treatments of the study. |
| Remarks field for test conditions (fill as applicable) | Test Organisms: Juvenile trout were obtained from Mount Lassen Trout Farms, Red Bluff, CA. At the end of the experiment the fish had an average wet weight of 1.2 g and an average length of 49.9 mm. The loading rate for the test was 0.80 g/L. Fish were held for 14 days prior to use in the test, and in the last two days prior to testing there was less than 3% mortality. Fish were fed during the holding period except for the 48 hours prior to use. Fish were not fed during the test.<br><br>Test System: Treatment levels were control and 1000 mg/L loading rate. Individual water accommodated fractions (WAFs) were prepared for each renewal of the 1000 mg/L treatment. Renewals occurred at 24-hour intervals. A measured weight of test material was added to a measured volume of dilution water (25 or 30-L) in a glass vessel and stirred for 20 hours. Stirring was accomplished using a magnetic stir bar. Mixing speed was adjusted such that a vortex formed that reached approximately 10% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 4 hours before the water phase was siphoned off. The siphoned water phase (i.e., WAF) was used in the aquatic toxicity test. The control and 1000 mg/L treatments consisted of three replicates each of 15 L test solution in 20L aquaria, which were lightly covered. Ten fish were used per replicate (thirty per treatment).<br>Dilution Water: Deionized water was collected at Marblehead, MA and adjusted to a hardness of 40-48 mg/L as $CaCO_3$. The water was continuously aerated and passed through a particle filter, ultraviolet |

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| | sterilizer and activated carbon during storage in a polyethylene tank. Water used for the test had a hardness of 44 mg/L as $CaCO_3$ and an alkalinity of 33 mg/L as $CaCO_3$.<br><br>Light: 16-h light per day using cool-white fluorescent lights with an intensity of approximately 4 μEin/$m^2$sec.<br><br>Test Temperature: 11.8 to 12.8 °C.<br><br>Water Chemistry: Dissolved oxygen: 7.1 – 10.2 mg/L, pH: 7.0 – 7.6, conductivity: 130 – 170 umhos/cm.<br><br>Element: Mortality<br><br>Test Levels: Control and 1,000 mg/L WAF loading rate.<br><br>Reference Substance: No |
| ***Results*** | Nominal concentrations: 24, 48, 72 and 96-h $LL_{50}$ (reported as "LC50" in the report) >1,000 mg/L. This is equivalent to 96-h $LL_0$ (reported as "NOEC" in the report) = 1,000 mg/L. |
| Remarks | Measured concentration: n/a<br>Analytical Monitoring: TOC levels at 0 hours were between 1.7 and 2.4 mg/L for the control and 2.0 and 3.6 mg/L for the 1,000 mg/L treatment. TOC levels at 24 hours were between 2.1 and 4.1 mg/L for the control and 2.4 and 3.7 mg/L for the 1,000 mg/L treatment. The active ingredient is in oil and TOC levels are not considered to be indicative of actual test material concentrations. Results are therefore based on nominal loading rates.<br><br>Unit: mg/L<br>Test Findings: Control survival was 100%, and 1000 mg/L treatment survival was 97%. No insoluble test material was observed in the test vessels during the entire aquatic toxicity test.<br><br>Other:<br>• Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates.<br>• Control response was satisfactory. |
| ***Conclusions*** | No significant mortality or signs of toxicity were observed in the 1000 mg/L WAF loading rate treatment or in the control throughout the entire test. |
| ***Data Quality*** | (1) Reliable without restriction |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 9-25-00 |
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**Robust Summary 26-Fish- 5**

| ***Test Substance*** | |
|---|---|
| CAS # | 1222384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts |
| Remarks | This substance is referred to as dodecyl sulfurized derivative in the HERTG's Test Plan for Alkyl Phenate Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Phenate Sulfide Category" in HERTG's Test Plan for Alkyl Phenate Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984). |
| Test Type | WAF static renewal test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1983 |
| Species/Strain | Fathead minnow (*Pimephales promelas*) |
| Analytical Monitoring | Total organic carbon (TOC) measurements of initial (0-h) test solutions and after one day on test (24-h) before renewal of fresh test solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1 |
| Exposure Period (unit) | 96 hours |
| Statistical methods | Statistical analysis of survival data not warranted. |
| Remarks field for test conditions (fill as applicable) | Test Organisms: Acquired from Aquatic Research Organisms, Hampton, New Hampshire, age: juvenile, total length: 30 mm average (longest fish not more than twice the shortest fish), wet weight: 0.2 g average (no range reported). Loading: <0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test.<br><br>Test System: Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (30-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used in the aquatic toxicity test. About 80% of the solution in each test level was renewed daily after 24, 48, and 72 hours. Two 15-L replicates per treatment, 10 fish per replicate (20 per treatment). Test vessels loosely covered to reduce entry of dust, etc.<br><br>Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. The water was characterized as moderately hard water. |

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| | Light: 16-h light per day using cool-white fluorescent lights with an intensity of 20 $uEin^{-1}$ $m^{-2}$.<br><br>Test Temperature: 21.0 to 22.8 C.<br><br>Water Chemistry: Dissolved oxygen: 7.4 – 8.5 mg/L, pH: 7.0 - 8.2, conductivity: 860 - 900 umhos/cm. Alkalinity not reported.<br><br>Element: Mortality<br><br>Test Levels: Control, 100, 300 & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test.<br><br>Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test.<br><br>Calculation of $LL_{50}$ s: Statistical analysis of survival data not warranted.<br><br>Analytical Monitoring: TOC levels were between 2.4 - 2.9 mg/L in the control, 2.7 - 3.0 mg/L at 100 mg/L test level, between 3.0 - 3.2 mg/L at 300 mg/L test level and 3.9 - 4.0 mg/L at the 1,000 mg/L test concentration level. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.<br><br>Reference Substance: No |
| ***Results*** | Nominal concentrations: 96-h $LL_{50}$ >1,000 mg/L. 96-h $LL_0$ = 1,000 mg/L. No mortality or toxic signs noted. |
| Remarks | Measured concentration: n/a<br><br>Unit: mg/L<br><br>LC50, LC0, LL50 or LL0 at 48, 72, 96-hours: $LL_{50}$ and $LL_0$ reported as $LC_{50}$ and NOEC, respectively, although test results were based on WAF loading rate.<br><br>Statistical results: Statistical analysis of survival data not warranted.<br><br>Other:<br>• Effect concentrations based on nominal loading rates.<br>• Control response was satisfactory. |

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| ***Conclusions*** | No mortality or signs of toxicity were observed in any of the treatments (100, 300 and 1,000 mg/L WAF loading rates) or in the control throughout the entire test. |
| ***Data Quality*** | Reliable without restrictions |
| ***References*** | This study is being submitted by the HERTG panel of the Chemical Manufacturers Association.<br><br>Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 608 to The Fathead Minnow, *Pimephales promelas.* T.R. Wilbury Study #9176-CMA/ESI-608. |
| ***Other*** | Updated: 2-2-00 |
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## 3.2 Acute Toxicity to Aquatic Invertebrates (e.g., Daphnia & Brown Shrimp)

### Robust Summary 26-Aquatic Invertebrates Tox - 1

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| ***Test Substance*** | |
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | U.S. EPA 797.1300 (1985, 1987), OECD 202 (1984) |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Yes |
| Year (Study Performed) | 1993 |
| Species/Strain | Daphnia magna |
| Test details (static, semi-static, dosing rate, flow-through rate, etc.) | A static non-renewal test was conducted using water accommodated fractions (WAF) of the test material at 100, 300 and 1,000 mg/L loading rates. WAFs were prepared by adding a measured weight of the test material to a measured volume of the dilution water and stirring for 24 hours with a magnetic stir bar. The test solutions were allowed to stand for 1 hour before the water phase (WAF) was siphoned off. |
| Statistical Methods | Not conducted because there was greater than 50% survival in all test vessels. |
| Remarks field for test conditions (fill as applicable) | Test species: Juvenile daphnids, less than 24-hours old were produced from laboratory in-house culture<br><br>Test conditions: Two 250-mL glass beakers that contained 200 ml of test solution were used per treatment. The 250-mL test vessels were loosely covered to reduce entry of dust, etc.<br><br>Test temperature range: 20 $\pm$ 1ºC<br><br>Exposure vessel type:<br><br>Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 168 to 172 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test, and 10 mg/L TSS at the beginning and <10 mg/L at the end of the test.<br><br>Lighting: A 16 hour light and 8 hour dark photoperiod was maintained with cool-whit fluorescent lights with an intensity of 20 $\mu Ein^{-1}m^{-2}$.<br><br>Water chemistry: Dissolved oxygen – 8.2 to 8.7 mg/L; pH – 7.8 to 8.2; conductivity – 570 to 640 umhos/cm; temperature – 20.5 to 20.9°C.<br><br>Element: Immobilization |

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| | Test design: Control, 100, 300 & 1,000 mg/L WAF loading rates. 10 daphnids per replicate (20 per treatment).<br><br>Method of calculating mean measured concentrations: not applicable<br><br>Exposure period: 48 hours<br><br>Analytical monitoring: Total organic carbon (TOC) measurements of initial test solutions and control (0-hour) and at test termination (48-h). TOC levels were 2 mg/L in the control, 3 mg/L at the 100 mg/L and 300 mg/L test levels; and 4 to 5 mg/L at the 1000 mg/L test vessel. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates |
| ***Results*** | Nominal concentrations: 48-hour and 24-hour EC50 = >1,000 mg/L (based on nominal loading rates). 48-hour and 24-hour NOEC = 1,000 mg/L |
| Remarks | Measured concentration: N/A<br><br>Unit: mg/L<br><br>EC50, EL50, LC0, LL0 at 24, 48 hours: 48-hour and 24-hour EC50 = >1,000 mg/L (based on nominal loading rates). 48-hour and 24-hour NOEC = 1,000 mg/L<br><br>Statistical results: not applicable<br><br>• Effect concentrations based on nominal loading rates<br>• No immobilization seen at the highest test concentration of 1,000 mg/L (WAF)<br>• Control response was satisfactory. |
| ***Conclusions*** | The WAFs of the test material were not toxic to daphnids at the concentrations tested. Ninety-five to 100% survival occurred at all test concentrations. No sublethal effects were noted during the test. |
| ***Data Quality*** | Reliable without restrictions |
| ***References*** | Chemical Manufacturers Association, HERTG<br><br>Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #613 to the Daphnid, *Daphnia magna.* T.R. Wilbury Study #9178-CMA/ESI-613. |
| ***Other*** | Updated: 12-21-99<br>. |

**Robust Summary 26-Aquatic Invertebrates Tox – 2**

| ***Test Substance*** | |
|---|---|
| CAS # | 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts |
| Remarks | This substance is referred to as dodecyl sulfurized derivative in the HERTG's Test Plan for Alkyl Phenate Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Phenate Sulfide Category" in HERTG's Test Plan for Alkyl Phenate Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984). |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1993 |
| Species/Strain | ***Cladoceran*, Daphnia magna** |
| Analytical Monitoring | Total organic carbon (TOC) measurements of initial (0-h) test solutions and at test termination (48-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1. |
| Exposure Period (unit) | 48 hours |
| Statistical methods | Statistical analysis of data not warranted. |
| Remarks field for test conditions (fill as applicable) | Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.<br><br>Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.<br><br>Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. The 250-mL test vessels were loosely covered to reduce entry of dust, etc.<br><br>Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 20 $uEin^{-1}m^{-2}$.<br><br>Test temperature: 20.0 – 20.9 C<br><br>Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene |

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| | tank where it was aerated. TOC levels were 2 mg/L at the beginning and end of the test and <10 mg/L at the end of the test.<br><br>Water chemistry: Dissolved oxygen: 8.2 - 8.7 mg/L; pH: 7.0 - 8.4; conductivity: 860 - 900 umhos/cm.<br><br>Element: Immobilization<br><br>Test Levels: Control, 100, 300 & 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test.<br><br>Test Findings: At 24-hours 0, 10, 5, and 15% immobilization were reported for control, 100, 300 and 1,000 mg/L treatments. At 48-hours 0, 10, 15, and 25% immobilization were reported for control, 100, 300, and 1,000 mg/L, respectively.<br><br>Calculation of $EL_{50}$ s: Statistical analysis of survival data not warranted.<br><br>Exposure period: 48 hours<br><br>Analytical Monitoring: TOC levels were 2.3 - 2.9 mg/L in the control, 2.6 - 3.0 mg/l at 100, 2.7 - 3.2 mg/L at 300 mg/L, and 3.0 - 4.0 mg/L at 1,000 mg/L test concentration. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. |
| ***Results*** | Nominal concentrations: 48-h $EL_{50}$ >1,000 mg/L. 48-h $EL_0$ = 100 mg/L based on WAF loading rates. |
| Remarks | Measured concentration: n/a<br><br>Unit: mg/L<br><br>EC50, EC0, EL50 or EL0 at 24 & 48 hours: $EL_{50}$ and $EL_0$ reported as $EC_{50}$ and NOEC, respectively, although test results were based on WSF loading rate. 24 & 48-h $EC_{50}$ >1,000 mg/L.; 24 & 48-h NOEC = 100 mg/L.<br><br>Statistical results: Not applicable.<br><br>Other:<br>• Effect concentrations based on nominal loading rates.<br>• 25% immobilization/mortality seen at the highest test concentration of 1,000 mg/L (WAF)<br>• Control response was satisfactory. |

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| ***Conclusions*** | The WAFs of the test material were not toxic to daphnids at the concentrations tested. Percent survival was 100% in the control, 90% at 100, 85% at 300, and 75% at 1,000 mg/L |
| ***Data Quality*** | Reliable without restrictions |
| ***References*** | This study is being submitted by the HERTG panel of the Chemical Manufacturers Association.<br><br>Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #608 to the Daphnid, *Daphnia magna.* T.R. Wilbury Study #9178-CMA/ESI-608. |
| ***Other*** | Updated: 2-11-00 |

**Robust Summary 26-Aquatic Invertebrates Tox – 3**

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| ***Test Substance*** | |
| CAS # | 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's Test Plan for Alkyl Phenate Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Phenate Sulfide Category" in HERTG's Test Plan for Alkyl Phenate Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline 40 CFR Part 797 (1993), and OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984). |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1996 |
| Species/Strain | ***Cladoceran*, Daphnia magna** |
| Analytical Monitoring | Total organic carbon (TOC) measurements were taken of initial (0-h) test solutions and at test termination (48-h). Controls, lowest and highest treatment levels were measured. Water samples were not filtered prior to analysis. Samples were subjected to TOC analysis using EPA Method 415.1. |
| Exposure Period (unit) | 48 hours |
| Statistical methods | Statistical analysis of data not warranted. |
| Remarks field for test conditions (fill as applicable) | Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.<br>Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. Test solutions were not renewed during the test. To prepare the WAFs, a measured weight of test material was added to a measured volume of dilution water and stirred for 20 hours. Stirring was accomplished using a magnetic stir bar. Mixing speed was adjusted such that a vortex formed that reached approximately 25% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 4 hours before the water phase was siphoned from the mixing vessel. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.<br><br>Test conditions: Two 300-mL glass beakers (test vessels) containing 250 mL of test solution were used per treatment level. The test vessels were loosely covered to reduce entry of dust. 10 daphnids were used per replicate (20 per treatment).<br><br>Light: 16-hour light per day using cool-white fluorescent lights with an intensity of ~6 uEin/$m^2$sec.<br><br>Test temperature: 20.1 – 21.0 °C<br><br>Dilution water: Deionized tap water was collected at Marblehead, MA |

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| | and adjusted to a hardness of 160-180 mg/L as $CaCO_3$ and a pH <8 with HCl. The water was continuously aerated and passed through a particle filter, ultraviolet sterilizer and activated carbon during storage in a polyethylene tank. Water used for the test had a hardness of 160-164 mg/L as $CaCO_3$ and an alkalinity of 122 mg/L as $CaCO_3$, and it contained <0.01 mg/L residual chlorine.<br><br>Water chemistry: Dissolved oxygen: 7.4 - 8.5 mg/L; pH: 7.4 - 8.4; conductivity: 600 umhos/cm.<br><br>Element: Immobilization/mortality<br><br>Test Levels: Control, 130, 220, 360, 600, & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire test.<br><br>Calculation of $EL_{50}$ s: Statistical analysis of survival data not warranted.<br><br>Exposure period: 48 hours |
| ***Results*** | Nominal concentrations: the 24 and 48-h $EL_{50}$s (stated "EC50s" in report) >1,000 mg/L loading rate. The 24 and 48-h NOELR (stated as "NOECs" in report) = 1000 mg/L based on WAF loading rates. |
| Remarks | Measured concentration: n/a<br>Analytical Monitoring: At the start of the experiment, TOC levels were 1.2-1.5 mg/L in the control, 2.0-2.6 mg/L at 130 mg/L loading rate, and 1.7 mg/L at 1,000 mg/L loading rate. At the end of the experiment (48 hr), the TOC levels were 5.6-5.7 mg/L in the control, 5.5-6.0 mg/L at 130 mg/L loading rate, and 5.4 mg/L at 1,000 mg/L loading rate. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.<br><br>Unit: mg/L<br><br>Statistical results: Not applicable.<br>Test Findings: 100% survival in the controls through 48 hours. At 24 hours, one dead or immobilized organism was noted for both 130 mg/L treatments and one of the 600 mg/L treatments. No additional immobilization or mortality was noted at 48 hours. Therefore, survival in all treatments was ≥90%.<br><br>Other:<br>• Test results reported in original study as "effect concentrations" (EC) are reported in this summary as "effective loading" (EL), because test results are based on WAF loading rates and not measured concentrations. Similarly, the "no observed effect concentration (NOEC) in the report is presented as "no observed effect loading rate" (NOELR) in this summary.<br><br>• Control response was satisfactory. |

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| | • Significant immobilization observed in preliminary tests at ≤1000 mg/L was likely due to insoluble material in the test vessels due to improper WAF preparation. WAF preparation for the final test did not exhibit insoluble material, and immobilization was not observed. |
| ***Conclusions*** | The test material was not toxic to *Daphnia magna* at loading rates tested. Percent survival/unaffected organisms was 100% in the control, 90% at 130, 100% at 220, 100% at 360, 95% at 600 and 100% at 1,000 mg/L loading rates. |
| ***Data Quality*** | (1) Reliable without restriction |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 9-25-00 |

**Robust Summary 26-Aquatic Invertebrates Tox – 4**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 72275-86-6 |
| Chemical Name | Alkenes, C15-18 alpha, reaction products with sulfurized tetrapropenyl phenol, calcium salt, sulfurized |
| Remarks | Test material purity not provided. |
| ***Method*** | |
| Method/Guideline followed | Guidelines issued by the U.K. Ministry of Agriculture, Fisheries and Food, Durham-on-Crouch, U.K. |
| Test Type | 96 Hour Acute Toxicity to Brown Shrimp (Water soluble fraction, semi-static renewal) |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1989 |
| Species/Strain | Brown Shrimp (Crangon crangon) |
| Number | 10/concentration/replicate (20/concentration total) |
| Size | Average length 4.1 cm; average weight 1.54g |
| Nominal Test Substance Concentration Levels | 0 (control), 100, 500, 1,000 and 10,000 mg/l (water soluble fraction) tested in duplicate (nominal concentration) |
| Test Concentration Preparation | Test solutions (water soluble fractions) were prepared separately for each test concentration by adding an appropriate aliquot of test material in diluent water and stirring overnight at the test temperature. The water-soluble extract was then siphoned off prior to testing. |
| Exposure Period | 96 hours |
| Exposure Conditions | Static renewal (daily) test conditions. |
| Vehicle | None |
| Statistical Analysis | $LC_{50}$ values and their 95% confidence limits determined at each evaluation time point |
| Dose Range finding Study | No |
| Test Chambers | Glass aquaria containing 20-liters of test solution and covered with aluminum foil to reduce volatilization |
| Diluent Water | Synthetic sea water (Synthetica) aerated for at least 12 hours prior to use. |
| Water chemistry during exposures | Temperature: $14^{O}C$<br>Dissolved oxygen (mg $O_2$/L): 8.0-8.3<br>pH: 8.1-8.2 |
| Photoperiod | 16 hours of light, 8 hours of dark |
| Positive Control | None |
| Remarks field for test conditions | All shrimp were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 3, 6, 24, 48, 72, and 96 hours after initiation of test material exposure was recorded. Measurements of pH, dissolved oxygen and temperature were recorded daily in each test chamber. Chambers were aerated to give ≥80% air saturation. Chamber loading was at 0.77 grams of body weight/L. Shrimp were not fed during the test period. Once daily, surviving shrimp were temporarily removed from the exposure chambers by gentle netting. The shrimp were moved to a small volume of test medium, while each chamber was thoroughly cleaned. Each chamber was refilled with fresh test solution and the shrimp were returned to the chambers. |

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| ***Results*** | Cumulative mortality (number dead / % mortality) was as follows:<br><br>Exposure Level (WSF) (mg/L) \| 3 hours \| 6 hours \| 24 hours \| 48 hours \| 72 hours \| 96 hours<br>0 \| 0 \| 0 \| 0 \| 1/5% \| 2/10% \| 2/10%<br>100 \| 0 \| 0 \| 0 \| 2/10% \| 4/20% \| 4/20%<br>500 \| 0 \| 0 \| 0 \| 3/15% \| 5/25% \| 5/25%<br>1000 \| 0 \| 0 \| 0 \| 2/10% \| 2/10% \| 3/15%<br>10000 \| 0 \| 0 \| 1/5% \| 17/85% \| 20/100% \| 20/100%<br>$LC_{50}$ mg/L (WSF) \| >10000 \| >10000 \| >10000 \| 7200 \| 4200 \| 2600<br>N =20/exposure level (10/replicate)<br><br>At 48 hours the three surviving shrimp at the 10000 mg/L concentration were all moribund. No adverse clinical signs were observed in the other test groups. |
| ***Conclusions*** | Under the conditions of this study the 24 hour $LC_{50}$ was >10000mg/L (WSF). The 48, 72 and 96 hour $LC_{50}$s were 7200, 4200 and 2600 respectively (WSF). A no effect concentration was not established. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of exposure concentration. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 12/12/2003 |
| | |

## 3.3 Toxicity to Aquatic Plants (e.g. Algae)

**Robust Summary 26-Algae - 1**

| | |
|---|---|
| ***Test Substance*** | |
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984). |
| Test Type | WAF static non-renewal test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1994 |
| Species/Strain | Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum* |
| Element basis (# of cells/ml) | ~10,000 cells/ml |
| Exposure period/duration | 96 hours |
| Analytical monitoring | Total organic carbon (TOC) measurements of initial test solutions and control (0-hour) and at test termination (96-h). EPA Method 415.1 (1979). Water samples were passed through 0.45 micron filter prior to TOC analysis. |
| Statistical Methods | |
| Remarks field for test conditions (fill as applicable) | Test Organisms: source – T.R. Wilbury in-house culture originally purchased from the University of Texas at Austin algae collection.<br><br>Test System: Individual WAFs were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made<br><br>visually by means of direct microscopic examination with a hemocytometer. Cool-white fluorescent lights provided a light intensity of 47 to 50 uEin/$m^2$sec.<br><br>At the conclusion of the 96-h test a 0.5 mL subsample of test media from each 100 mg/L test flask was combined with 100 mL of fresh untreated alga media |

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| | and incubated for up to 9 days or as soon as growth occurs. This was done to determine if growth inhibition was algistatic or algicidal.<br><br>Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test temperature – 23.4 to 23.6 C, pH – 7.0 to 7.1 at 0-hour and 8.6 to 10.2 after 96 hours. TOC measurements were only made on the lowest and highest test levels and control at the beginning and end of the test. TOC levels were <1.0 mg/L in the control and 1.0 mg/L WAF test level and 3 mg/L at 100 mg/L.<br><br>Test Levels: Control, 1.0, 5.0, 10, 50, 100 mg/L WAF loading rates.<br><br>Calculation of $EL_{50}$ s and NOELRs: Moving average and probit methods (Stephan, 1983) were used to calculate $EC_{50}$s (i.e., $EL_{50}$s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect concentration (i.e., $EL_0$s) when data were normally distributed and a non-parametric Kruskal and Wallis test was used if data were not normally distributed.<br><br>Test Substance: No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test.<br><br>Reference Substance: No |
| ***Results*** | Measurements expressed as mg/L WAF loading rate:<br><br>72-h $EL_{50}$ / 72-h NOELR [c] / 96-h $EL_{50}$ / 96-h NOELR [c]<br>Cell Density: 26[a] (21-32) / 5.0 / 34[b] (29-39) / 10<br>Growth Rate: >100 / 5.0 / >100 / 10<br>[a] Moving average method. [b] Probit method.<br>Confidence limits in parentheses.<br>[c] Hypothesis analysis tests were used to determine NOELRs.<br><br>Re-growth of inhibited cultures from the 100 mg/L test level revealed the effect was algistatic rather than algicidal. |
| Remarks | Measured concentration: N/A<br><br>Unit: mg/L WAF loading<br><br>Element value: $EL_{50}$ and NOELR (i.e., no-observable effect loading rate).<br><br>• $EL_{50}$s and NOELRs reported as $EC_{50}$ and NOEC, respectively, although test results were based on WAF loading rates.<br>• Test concentrations for the definitive test were not specified in a protocol amendment and the pH of the sterile media at the start of the test was 7.0 rather than 7.5. These deviations did not compromise the study. |

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| ***Conclusions*** | Re-growth of inhibited cultures from the 100 mg/L test level revealed the effect was algistatic rather than algicidal. |
| ***Data Quality*** | Reliable without restrictions |
| ***References*** | Chemical Manufacturers Association, HERTG |
| ***Other*** | Updated: 12-27-99 |
| | |

**Robust Summary 26-Algae-2**

| ***Test Substance*** | |
|---|---|
| CAS # | 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide.<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenol sulfide and 40% highly refined lubricant base oil. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984). |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1994 |
| Species/Strain | ***Freshwater algae,* Pseudokirchneriella subcapitata *formerly called* Selenastrum capricornutum.** |
| Element basis (# of cells/mL) | $10^{+4}$ cells/mL |
| Exposure period/duration | 96 hours |
| Analytical monitoring | Total organic carbon (TOC) measurements of initial (0-h) high, low and control test solutions and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1. |
| Statistical methods | A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect concentration (i.e., $EL_0$s). |
| Remarks field for test conditions (fill as applicable) | Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection.<br><br>Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.<br><br>Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were |

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| | stoppered with foam plugs to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer.<br><br>Light: Cool-white fluorescent lights provided a light intensity of 47-50 uEin/$m^2$sec 24-h per day.<br><br>Test temperature: 23.4 to 23.7 C.<br><br>Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.5 - 7.8 at 0-hour and 9.5 - 10.6 after 96 hours.<br><br>Test Levels: Control, 125, 250, 500, 700, and 1000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test.<br><br>Calculation of $EL_{50}$ s and NOELRs: Moving average and probit methods (Stephan, 1983) were used to calculate $EC_{50}$s (i.e., $EL_{50}$s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect concentration (i.e., $EL_0$s) when data were normally distributed and a non-parametric Kruskal and Wallis test was used if data were not normally distributed.<br><br>Exposure period: 96 hours |
| ***Results*** | Nominal concentrations: 72- & 96-h $EL_{50}$>1,000 mg/L and 72- & 96-h NOELR = 1000 mg/L based on both growth rate and biomass measurements. |
| Remarks | Measured concentration: n/a<br>Analytical monitoring: At the beginning and end of the test, TOC measurements were non-detect (<1.0 mg/L) in control and 125 mg/L and 2.0 mg/L at 1,000 mg/L. The active ingredient is in oil and TOC levels are not considered to be indicative of actual test material concentrations. The results are therefore based on nominal loading rates.<br><br>Unit: mg/L<br><br>Test Findings: At 72-hours biomass measurements in the treatments were between 74 (at 1,000 mg/L) to 123% of the control. At 96-hours they were between 93 - 109 % of the control.<br><br>Other:<br>• Effect concentrations based on nominal loading rates.<br>• Test results reported in original study as "effect concentrations" (EC) are reported in this summary as "effective loading" (EL), because test results are based on WAF loading rates and not |

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| | measured concentrations. Similarly, the "no observed effect concentration (NOEC) in the report is presented as "no observed effect loading rate" (NOELR) in this summary.<br>• Control response was satisfactory. |
| ***Conclusions*** | The WAFs of the test material were not toxic to freshwater alga at concentrations up to and including 1,000 mg/L. |
| ***Data Quality*** | (1) Reliable without restrictions. |
| ***References*** | Ward, T.J. (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #608 to the Freshwater Alga, *Selenastrum capricornutum.* T.R. Wilbury Study #73-CM-608. |
| ***Other*** | Updated: 2-11-00 |

**Robust Summary 26-Algae-3**

| ***Test Substance*** | |
|---|---|
| CAS # | 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide.<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenol sulfide and 40% highly refined lubricant base oil. |
| ***Method*** | |
| Method/Guideline followed | Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1993), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984). |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1997 |
| Species/Strain | ***Freshwater algae,*** **Pseudokirchneriella subcapitata** ***formerly called*** **Selenastrum capricornutum** |
| Element basis (# of cells/mL) | approximately 10,000 cells/mL |
| Exposure period/duration | 96 hours |
| Analytical monitoring | Total organic carbon (TOC) measurements of initial (0-h) high, low and control test solutions and at test termination (96-h). Water samples were not filtered prior to TOC analysis using EPA Method 415.1 |
| Statistical methods | Probit methods (Stephan, 1983) were used to calculate EC (EL) values ($EL_{10}$ s, $EL_{50}$ s and $EL_{90}$ s). A parametric one-way analysis of variance (ANOVA) was used to calculate the no-observed effect level rate (NOELR). |
| Remarks field for test conditions (fill as applicable) | Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection.<br><br>Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. Test solutions were not renewed during the test. To prepare the WAFs, a measured weight of test material was added to a measured volume of dilution water and stirred for 20 hours. Stirring was accomplished using a magnetic stir bar. Mixing speed was adjusted such that a vortex formed that reached approximately 25% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 4 hours before the water phase was siphoned from the mixing vessel. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.<br><br>Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks (test vessels) were covered with inverted glass beakers to reduce entry of dust, etc. During the test all treatment and control flasks were |

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| | randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer.<br><br>Light: Cool-white fluorescent lights provided a light intensity of 360-370 footcandles.<br><br>Test temperature: 23.5 to 23.7 °C.<br><br>Dilution Water: Sterile enriched alga growth media (US EPA, 1978) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were 1.2 and <10 mg/L, respectively.<br><br>Test Levels: Control, 130, 220, 360, 600, and 1000 mg/L WAF loading rates. No insoluble test material was seen in any of the test vessels during the entire aquatic toxicity test.<br><br>Water chemistry: pH: initial = 7.4 – 7.5, final = 9.2 – 10.6<br><br>Method of calculating mean measured algal cell densities: microscopic examination using a haemocytometer.<br><br>Exposure period: 96 hours. |
| ***Results*** | No unusual cell shapes, flocculations, etc. noted during the test. The 96 hour EL50 (presented as "EC50" in report) for both cells per mL and growth rate is >1,000 mg/L nominal WAF loading rate. The 96 hour NOELR (presented as "NOEC" in report) is 360 mg/L nominal WAF loading rate as calculated using the number of cells per mL, and 220 mg/L nominal WAF loading rate as calculated using average specific growth rate. Effects were determined to be algistatic rather than algicidal. |
| Remarks | Measured concentration: n/a<br>Analytical monitoring: At the beginning (0 hr) and end (96 hr) of the test, TOC measurements were: 1.1-1.2 and 2.5-2.6 mg/L in control, 1.2-1.8 and 2.1-2.7 mg/L at 130 mg/L WAF loading rate, and 2.5-2.7 and 2.3-2.4 mg/L at 1,000 mg/L WAF loading rate. The active ingredient is in oil and TOC levels are not considered to be indicative of actual test material concentrations. The results are therefore based on nominal WAF loading rates.<br><br>Unit: mg/L<br><br>Algistatic effects were observed in the first 24 hour period; after this the growth rate of the algae in the 1,000 mg/L WAF treatment appeared to be similar to the other treatment levels (and control).<br><br>Other:<br>• Test results reported in original study as "effect concentrations (EC)" and "no observed effect concentrations (NOEC)" are |

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| | reported in this summary as "effect loading (EL)" and "no observed effect loading rate (NOELR)", respectively, because test results are based on nominal WAF loading rates.<br>• Control response was satisfactory. |
| ***Conclusions*** | The test material was not algicidal to freshwater alga at loading rates up to and including 1,000 mg/L, but the material had an algistatic effect at approximately 200 to 300 mg/L WAF loading rate. |
| ***Data Quality*** | (1) Reliable without restriction |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 9-26-00 |

## 4.0 MAMMALIAN TOXICITY

### 4.1 Acute Oral Toxicity

**Robust Summary 26-Acute Oral –1**

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|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 72275-86-6 |
| Chemical Name | Alkenes, C15-18 alpha, reaction products with sulfurized tetrapropenyl phenol, calcium salt, sulfurized |
| Remarks | Test material dosed as received, purity not provided. |
| ***Method*** | |
| Method/Guideline followed | FHSA 16CFR1500.3 |
| Test Type | Acute oral toxicity |
| GLP (Y/N) | N |
| Year (Study Performed) | 1980 |
| Species/Strain | Rats/ Sprague-Dawley strain |
| Sex | Male/Female |
| No. of animals/dose | 5/sex |
| Vehicle | None |
| Route of administration | Oral (intragastric) |
| Dose level | 5 g/kg |
| Control group included | Yes |
| Remarks field for test conditions | A single dose of the test material was administered intragastrically to five fasted male and female rats. The animals were observed for signs of toxicity or behavioral changes on the day of dosing and twice daily thereafter. Individual weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized after 14 days. Necropsies were performed on all animals. Body weights were evaluated statistically using the Student t-test. |
| ***Results*** | LD50 > 5 g/kg (males and females) |
| Remarks | All animals survived the duration of the study. No signs of toxicity were observed during the study. Body weights were unremarkable. At necropsy, one female control exhibited red urine in the bladder and a hollow kidney. No pathological treatment related findings were observed. |
| ***Conclusions*** | The test article, when administered to male and female Sprague-Dawley rats, had an acute oral LD50 of >5 g/kg. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code). |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 3/04/2003 |
| | |

**Robust Summary 26-Acute Oral –2**

| ***Test Substance*** | |
|---|---|
| CAS # | 122384-85-4 |
| Chemical name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide.<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenol sulfide and 40% highly refined lubricant base oil. |
| ***Method*** | |
| Method/guideline followed | Consistent with OECD 401 |
| Test type | Acute oral toxicity |
| GLP (Y/N) | Y |
| Year (study performed) | 1996 |
| Species/strain | Rat, Sprague-Dawley, Crl:CD® (SD)BR, age 63-70 days at initiation of treatment |
| Sex | Male and female |
| No. of animals/sex/dose | 5 |
| Vehicle | Corn oil |
| Route of administration | Oral gavage |
| Remarks field for test conditions | The test material was diluted in corn oil to a concentration of 0.5 g/mL and administered at a limit dose of 5000 mg/kg b. wt. The animals were held for a 14-day observation period. The animals were observed at 1, 2.5, and 4 hours post-exposure on the day of dosing and twice daily thereafter. Individual body weights were recorded prior to dosing and on days 7 and 14 after dosing. The animals were euthanized by $CO_2$ inhalation at the conclusion of the observation period and examined for macroscopic pathological changes. Tissues with macroscopic abnormalities were preserved for microscopic examination.<br><br>All guideline recommendations were exceeded. |
| ***Results*** | No mortality at 5000 mg/kg b. wt. |
| Remarks | All animals survived to termination of the experiment. Most animals showed no clinical signs of toxicity until 4 hour after exposure, when 3 males and 1 female had soft stool. Between days 1 and 4 most animals exhibited dark-stained urogenital area and/or red stained face. All animals appeared normal by day 7 post-exposure. All animals gained body weight at both 7 and 14 days post-exposure. No macroscopic abnormalities were observed at termination. |
| ***Conclusions*** | $LD_{50}$ > 5000 mg/ kg b. wt. (males and females) |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 5 Mar 2000 |

**Robust Summary 26-Acute Oral –3**

| | |
|---|---|
| ***Test Substance*** | |
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Experimental |
| Test Type | Acute oral toxicity (LD50) |
| GLP (Y/N) | N |
| Year (Study Performed) | 1970 |
| Species/Strain | Rat; Sherman/Wistar |
| Sex | Male, young adult |
| No. of animals/sex/dose | 5 |
| Vehicle | None; administered undiluted |
| Route of administration | Oral gavage |
| Remarks field for test conditions | Rats fasted 24 hours prior to dosing; Test material administered by gavage in a single oral dose at concentrations of 2.0, 4.0, 8.0, 16.0 or 32.0 ml/kg. Animals observed for 14 days postdosing for signs of toxicity or mortality . Body weights were not taken; gross necropsies and histopathology were not performed |
| ***Results*** | 5.7 ml/kg; 19/20 confidence limits 4-8 ml/kg |
| Remarks | No deaths were observed at 2.0 or 4.0 ml/kg; at 8 ml/kg 4/5 dead at day 1 post dosing , 1/5 dead at day 2; 5/5 rats dead at day 1 in groups given 16.0 or 32.0 ml/kg. No data presented on parameters other than mortality. |
| ***Conclusions*** | LD50 = 5.7 ml/kg (male rats) |
| ***Data Quality*** | Reliable with restrictions<br>State of the art LD50 oral toxicity study for 1970, multi-dose, calculated LD50 with confidence limits. Non-GLP, details of study design and observations during study not presented |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-29-99 |
| | |

**Robust Summary 26-Acute Oral –4**

| ***Test Substance*** | |
|---|---|
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Litchfield and Wilcoxen (J. Pharm. & Exp. Therap. 96:99, 1949) |
| Test Type | Acute oral toxicity (LD50) |
| GLP (Y/N) | N |
| Year (Study Performed) | 1979 |
| Species/Strain | Rat/Wister |
| Sex | Male |
| No. of animals/sex/dose | 40 (4 groups of 10) |
| Vehicle | None: administered undiluted |
| Route of administration | Oral gavage |
| Remarks field for test conditions | Rats fasted for 24 hours prior to dosing; Test material administered by gavage in a single oral dose at concentrations of 5.0, 7.12, 10.14, and 14.43 g/kg. Animals observed 3-4 hours after dosing and once daily for 14 days post-dosing. Mortality, toxicity, and pharmacological effects were recorded for each animal. Body weights were recorded pretest. At the end of 14 days, all survivors were sacrificed and all animals, including those which died during the course of the study, were examined for gross pathology. |
| ***Results*** | LC50 = (6.8 – 10.9)g/kg |
| Remarks | At 5.0 g/kg 2/10 died (1 at day 1 post dose and 1 at day 13 post dose); at 7.12 g/kg 4/10 died (3 at day 2 post dose and 1 at day 6 post dose); at 10.14 g/kg 6/10 died at day 2 post dose; at 14.43 g/kg 10/10 died (1 at day 1 post dose and 9 at day 2 post dose). Toxicity was observed for the following doses: at 5.0 g/kg lethargy, ataxia, ptosis, piloerection and flaccid muscle tone were noted in 5 or more animals. Isolated instances of diarrhea, chromorhinorrhea, chromodacryorrhea, and tachypnea were also noted; at 7.12 g/kg lethargy, diarrhea, piloerection, ptosis, chromodacryorrhea, ataxia, and chromorhinorrhea were noted in 5 or more animals. Isolated instances of tachypnea, respiratory noise and prostration were also noted; At 10.14 g/kg lethargy, diarrhea, chromorhinorrhea, ptosis, piloerection, chromodacryorrhea and ataxia were noted in 5 or more animals. Isolated instances of emaciation, prostration and hyperactivity were also noted; at 14.43 g/kg lethargy, ataxia, ptosis, diarrhea, chromorhinorrhea and piloerection were noted in 5 or more animals. Overall body weights increased slightly in the 5.0, 7.12, and 10.14 g/kg doses. No body weights were reported for the 14.43 g/kg dosed animals. Necropsy was also performed. |

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|---|---|
| ***Conclusions*** | LD50 = 8.6 g/kg (male rats) |
| ***Data Quality*** | Reliable with restrictions<br>State of the art LD50 oral toxicity study for 1979, multidose, calculated LD50, no reference to confidence limits. Non-GLP, details of the study design and other observations not presented. Used 16 CFR 1500.3(c) (2) (I) for toxicity definitions. |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-28-99 |
| | |

## 4.2 Acute Dermal Toxicity

**Robust Summary # 26-Acute Dermal-1**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 72275-86-6 |
| Chemical Name | Alkenes, C15-18 alpha, reaction products with sulfurized tetrapropenyl phenol, calcium salt, sulfurized |
| Remarks | Test material purity not provided. |
| ***Method*** | |
| Method/Guideline followed | OECD Guideline 402 |
| Test Type | Acute dermal toxicity (Limit Test) |
| GLP (Y/N) | N |
| Year (Study Performed) | 1980 |
| Species/Strain | New Zealand Albino Rabbits |
| Sex | Male and female |
| No. of animals/sex/group | 5 |
| Vehicle | None |
| Route of administration | Dermal |
| Dose level | 5 g/kg |
| Dose volume | Not specified |
| Control group included | Yes |
| Remarks field for test conditions | Approximately 24 hours prior to topical application of the test material, the hair of each control and treated animal was closely clipped. Immediately prior to dosing, the back of each rabbit was abraded with a hypodermic. (Note: This study was conducted several years before OECD Test Guideline 402 was adopted. The test guideline does not call for the use of abraded skin. This deviation from the guideline was not considered significant.) A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and five female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a plastic sheet wrapped around the animal's trunk. Control animals were also clipped, abraded and wrapped. Collars were placed on the animals for 14 days to prevent oral ingestion of the test material. After the 24-hour exposure period the wrappings and any remaining test material were removed from the animals. The animals were observed for 14 days after treatment. Individual body weights were recorded prior to dosing at 7 days and at study termination. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14. A section of skin from each animal was examined histopathologically. Body weights were evaluated statistically using the Student t-test. |
| ***Results*** | LD50 > 5.0 g/kg (males and females) |
| Remarks | All animals survived the duration of the study. During the exposure period the test material caused severe edema; erythema was obscured due to skin discoloration caused by the test material. Decreased food consumption and depression were observed in some treated animals. At seven days, moderate to severe erythema and edema were noted and the skins were hard and thickened. Male body weights were significantly lower than control at 7 and 14 days. Except for thickened and flaky skin observed at necropsy, no other gross pathological |

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| | changes were attributed to the test material. Histopathological evaluation of skin sections revealed diffuse acanthosis, diffuse hyperkeratosis and diffuse and multifocal subacute dermatitis. These findings were all considered reversible. |
| ***Conclusions*** | The test article, when administered dermally as received to 5 male and 5 female New Zealand Albino Rabbits had an acute dermal LD50 of greater than 5.0 g/kg. Dermal exposure to the test material was associated with severe edema, erythema, skin discoloration decreased food consumption, depression and reduced body weights (males only). Skin sections exhibited diffuse acanthosis; diffuse hyperkeratosis and diffuse and multifocal subacute dermatitis. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the failure of the report to include individual animal dermal observation data. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 3/05/2003 |
| | |

**Robust Summary # 26-Acute Dermal-2**

| | |
|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 67762-55-4 |
| Chemical Name | Alkenes, C15-18 alpha, sulfurized |
| Remarks | This chemical is also referred to as C15-C18 alkene derivative in the HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | |
| Test Type | Acute dermal toxicity, single exposure |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1996 |
| Species/Strain | New Zealand White rabbits |
| Sex | Male and female |
| No. of animals/sex/dose | 10/dose (5M, 5F) |
| Vehicle | None, test article was doses as received. |
| Route of administration | Dermal, to clipped intact, dorsal skin |
| Remarks field for test conditions | One dermal, semi-occluded patch of test article at 2,000 mg/kg was applied to clipped dorsa skin of each animal. The patches were removed after 24 hours. All animals were observed daily for 14 days following test article administration. |
| ***Results*** | |
| Remarks | No clinical signs were observed during the study. Erythema and/or edema of the skin at application site were observed on Day 1 in some animals. There was an increase in mean body weight during the study. None of the animals died during the study. No visible lesions were observed in any animal at terminal necropsy. |
| ***Conclusions*** | LD50 > 2000 mg/kg |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-29-99 |
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**Robust Summary # 26-Acute Dermal-3**

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|---|---|
| ***Test Substance*** | |
| CAS # | CAS # 122384-85-4 |
| Chemical name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | Testing was performed a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenate sulfide and 40% highly refined lubricant base oil.<br><br>This chemical is also referred to as dodecyl derivative in the HERTG's Test Plan for Alkyl Phenate Sulfide Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Phenate Sulfide Category" in HERTG's Test Plan for this Category. |
| ***Method*** | |
| Method/guideline followed | Consistent with OECD 402 |
| Test type | Acute dermal toxicity |
| GLP (Y/N) | Y |
| Year (study performed) | 1996 |
| Species/strain | Rat, Sprague-Dawley, Crl:CD® (SD)BR, age 49-77 days at initiation of treatment |
| Sex | Male and female |
| No. of animals/sex/dose | 5 |
| Vehicle | None |
| Route of administration | Semi-occluded topical application |
| Remarks field for test conditions | The test material was administered undiluted to shaved, intact skin (approximately 36 $cm^2$) at a limit dose of 2000 mg/kg b. wt. and covered with a semi-occlusive dressing. After 24 hours the dressing was removed and the site washed with mineral oil followed by a mild soap solution. The animals were held for 14 days and observed at 1, 2.5, and 4 hours after test material application and twice daily thereafter. Dermal irritation was scored 30 minutes after dressing removal and on days 3, 7, 10 and 14. Individual body weights were recorded prior to dosing and on days 7 and 14 after dosing. The animals were euthanized by $CO_2$ inhalation at the conclusion of the observation period and examined for gross pathological changes. Tissues with macroscopic abnormalities were preserved for microscopic examination.<br><br>All guideline recommendations were exceeded. |
| ***Results*** | No mortality at 2000 mg/kg b. wt. |
| Remarks | All animals survived to termination of the experiment. All animals appeared normal throughout the observation period. Slight erythema was observed in one female and one male on day 1. Full recovery occurred in the female by day 3 and in the male by day 7. All animals gained body weight at both 7 and 14 days post-exposure, except for a female that gained no weight the first week and another female that lost 6 grams during the second week. No tissues were observed with macroscopic abnormalities at termination. |
| ***Conclusions*** | $LD_{50}$ > 2000 mg/ kg b. wt. (males and females) |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 5 Mar 2000 |

**Robust Summary # 26-Acute Dermal-4**

| | |
|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide.<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenol sulfide and 40% highly refined lubricant base oil. |
| ***Method*** | |
| Method/Guideline followed | 21 CFR 191.10 |
| Test Type | Acute Dermal Toxicity |
| GLP (Y/N) | N |
| Year (Study Performed) | 1972 |
| Species/Strain | New Zealand White |
| Sex | Unspecified |
| No. of animals | Six |
| Vehicle | None |
| Route of administration | Dermal |
| Dose level | 15,000 mg/kg |
| Vehicle control group | None |
| Chemical analysis of dosing solution | No |
| Remarks field for test conditions | The test material was applied to the closely clipped trunk of three abraded and three unabraded rabbits. The test material was applied undiluted to the backs under a plastic sleeve that was wrapped around the entire trunk of the animal. Paper towels were wrapped over the plastic to prevent tearing. The sites were uncovered after 24 hours. The animals were observed for gross signs of systemic toxicity daily for 14 days. Necropsies were performed. |
| ***Results*** | LD50 >15,000 mg/kg |
| Remarks | All animals survived the duration of the study. No abnormal clinical signs were evident. The 24-hour exposure to the test material produced a slight to moderate degree of dermal irritation. No gross pathological changes were evident with the exception of dry and flaky skin at the treatment sites. |
| ***Conclusions*** | The test article, when administered to 6 rabbits, had an acute dermal LD50 of >15,000 mg/kg. Slight to moderate dermal irritation was evident in all animals. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code). |
| ***References*** | Unpublished confidential business information |
| ***Other*** | October 7, 2002 |
| | |

## 4.3 Repeated Dose Toxicity

### Robust Summary 26-Repeated Dose-1

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|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 67124-09-8 |
| Chemical Name | 2-propanol, 1-(tert-dodecylthio)- |
| Remarks | 100% purity<br>This chemical is also referred to as propanol/dodecylthio derivative in the HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | OECD 407 |
| Test Type | 28-day oral toxicity study in rats |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1991 |
| Species | Rat |
| Strain | Sprague-Dawley CD, 41 days old at initiation of treatment |
| Route of administration | Oral gavage (syringe and dosing tube) |
| Duration of test | 28 days of treatment and 14 day recovery period in the control and high dose satellite recovery groups |
| Doses/concentration levels | 0, 100, 300 and 1000 mg/kg/day |
| Sex | Males and females |
| Exposure period | 28-day treatment duration with a 14 day recovery |
| Frequency of treatment | 7 days/week |
| Control group and treatment | 5 rats/sex/group for each dose, and satellite recovery groups of 5 animals/sex for the control and 1000 mg/kg/day dose. Control group received daily doses of corn oil at 2.0 ml/kg, and treatment groups received the indicated dose of test material diluted in corn oil in a volume not to exceed 2.0 ml/kg |
| Post exposure observation period | 14-days |
| Statistical methods | Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's *post-hoc* test, non-parametric Kruskal-Wallis and Dunn's rank sum test, Bartlett's test for equal variances, and Student's *t*-test. |
| Remarks field for test conditions | Significant deviations from the OECD 407 test guidelines include:<br>• A function observational battery for neurotoxicity was not performed since this test was not part of the OECD 407 guideline at the time the study was performed |
| ***Results*** | |
| Remarks | No NOAEL was assigned to this study.<br>All animals survived throughout the study and physical examinations were generally unremarkable. Test material administration produced alterations in the liver and kidneys of treated animals that were evident in the evaluation of organ weights as well as gross and microscopic pathological examinations.<br>Dose-related elevations in mean liver weights and/or liver/body weight |

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| | ratios were seen at study termination in males at all dose levels and in females at the mid- and high-dose levels. Recovery was apparent during the two-week recovery period for the high-dose group. Gross post mortem examination of the liver revealed an accentuated lobular pattern in the mid- and high-dose females at termination of the dosing period, which resolved during the recovery period. Microscopic examination of liver revealed hepatocyte hypertrophy in all dose groups at the termination of treatment. This effect continued through the recovery period. The effect on the liver was consistent with the adaptive induction of hepatic metabolic mechanisms in response to a xenobiotic challenge.<br>Kidney alterations were seen only in males. Kidney weights and kidney/body weight ratios for high-dose males were significantly higher than control values at termination of dosing. These values were comparable following termination of the recovery period. Gross post mortem examination of the kidneys revealed pale or tan discoloration of increasing frequency with increased dose. Microscopic alterations consisted of increased incidences of globular casts and hyaline droplets in treated males. Hyaline droplets in the proximal tubules were seen at termination of dosing only, indicating that this change in renal morphology was reversible after cessation of test substance administration. The renal effects are consistent with previous reports in the scientific literature of male rat-specific hydrocarbon nephropathy. Evaluation of clinical chemistry and urinalysis studies revealed no evidence of renal or hepatic functional alterations, or any other signs of systemic effects due to the test material. Other minor effects of the test material consisted of a transient decrease in food consumption and body weight gain in the high-dose male group during the first week of study. A slight decrease in hemoglobin and hematocrit values was observed in the high-dose female group at termination that was found to be reversible during the 2-week recovery period. |
| ***Conclusions*** | Although renal and hepatic changes were evident at all dose levels (100, 300, and 1000 mg/kg/day), the renal changes are species-specific and the hepatic changes are probably adaptive in nature. Therefore, little subchronic toxicity was observed over the range of doses administered in this study. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-27-99 |
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**Robust Summary 26-Repeated Dose-2**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS # 122384-85-4 |
| Chemical Name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | Testing was performed a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenate sulfide and 40% highly refined lubricant base oil.<br><br>This chemical is also referred to as dodecyl derivative in the HERTG's Test Plan for Alkyl Phenate Sulfide Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Phenate Sulfide Category" in HERTG's Test Plan for this Category. |
| ***Method*** | |
| Method/Guideline followed | OECD 422 (draft) |
| Test Type | 4-week repeated dose oral toxicity study in rats |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1996 - 1997 |
| Species | Rat |
| Strain | Sprague-Dawley, Crl:CD® BR, age 45 days at initiation of treatment |
| Route of administration | Oral gavage |
| Duration of test | 30 days for all dose groups and 45 days for the control and high dose recovery groups |
| Doses/concentration levels | 0, 50, 300 and 1000 mg/kg b.wt./day administered in a dose volume of 5 mL/kg b.wt./day |
| Sex | Males and females |
| Exposure period | 29 days |
| Frequency of treatment | Daily |
| Control group and treatment | 6 rats/sex/group for all control, test and recovery groups. Control rats received corn oil vehicle only |
| Post exposure observation period | 15 days for the control and high dose recovery groups |
| Statistical methods | Body weight, food consumption, feed efficiency, hematology, serum chemistry, urinalysis, and organ weight data were analyzed with Bartlett's test to determine homogeneity of variances. If group variances were equal, data were analyzed by ANOVA with a Dunnett's test if significance was observed with ANOVA. If group variances were unequal, data were analyzed by the non-parametric Kruskal-Wallis test with a Dunn's rank sum test if significance was observed with Kruskal-Wallis. A test for dose-response trend was also conducted using regression analysis for parametric data and Jonckeere's test for monotonic trend for non-parametric data. . Statistical analysis was not performed when the standard deviation of the control group was zero and dose groups were eliminated when their standard deviation was zero. If only one dose group remained, they were analyzed with a standard t-test if variances were equal, or Welch's t-test if variances were unequal. |
| Remarks field for test conditions | Corn oil dosing solutions were prepared weekly, and their test material concentration, homogeneity and stability verified by chemical analysis. There were 6 rats/sex/group for the 4-week exposure groups and for the control and high dose 2-week recovery groups. In addition there were control and high dose satellite groups of 12 males for |

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| | neurotoxicity evaluations. Animals were observed twice daily (morning and afternoon) and one hour after dosing during the administration period for mortality or moribundity. Detailed clinical observations were recorded weekly. Individual body weights and food consumption were recorded twice pre-study and then weekly during the exposure and recovery periods, and at termination. A functional observation battery (home cage, handling, open field, sensory, neuromuscular and physiological observations) was conducted pre-study and on days 14, and 28 with 12 males of the control and high dose satellite groups and on day 42 (recovery) with 6 males of the control and high dose satellite groups. After the exposure and recovery periods, main study animals were fasted while urine samples were collected overnight, then euthanized with carbon dioxide. Urine volume was measured as well as urinalysis parameters. Blood samples were collected from all main study animals at necropsy for determination of hematology and serum chemistry parameters. Six control and high dose satellite males were treated with whole-body perfusion at the end of the exposure and recovery periods for subsequent microscopic examinations on extensive central and peripheral nervous system tissues including eyes. Complete gross necropsy examinations were performed on all main study animals. Eleven major organs were weighed and 27 organs / tissues were preserved for all main study groups. Microscopic observations were made on these tissues only if macroscopic lesions were found.<br><br>All guideline recommendations were exceeded, except for the following: Functional observations were not obtained for females. Heart weights were not measured. |
| ***Results*** | |
| NOAEL (NOEL) | NOAEL = 300 mg/kg b.wt./day for males and females |
| LOAEL (LOEL) | LOAEL = 1000 mg/kg b.wt./day for males and females based on increased adrenal weights and increased food consumption without commensurate body weight gain |
| Actual dose received | 0, 50, 300 and 1000 mg/kg b.wt./day |
| Toxic effects | Increased adrenal weights and increased food consumption without commensurate body weight gain in males and females |
| Statistical results | Food consumption was significantly ($p \leq 0.01$) elevated in high-dose males and females during the treatment period. Mean blood cholesterol values for the mid- and high-dose males were significantly decreased ($p \leq 0.05$ and $p \leq 0.01$ respectively) compared to controls after exposure. Adrenal weights and weight ratios for high-dose females at the end of the exposure period and for high-dose males at the end of the recovery period were significantly elevated ($p \leq 0.05$ or $p \leq 0.01$) compared to respective controls. There were no other statistically significant differences of toxicological significance |
| Remarks | The homogeneity and stability of test material in corn oil dosing solutions were confirmed by chemical analysis of calcium content. Measured concentrations of test material in dosing solutions collected during the study were within 15% of nominal concentrations. **Survival:** All animals survived to termination. **Clinical observations:** No treatment-related clinical signs were observed. **Functional observation battery:** There were no differences between observations made for high-dose and control males during exposure or after |

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| | recovery. **Body weights and food consumption:** Body weight and food consumption data of main study and satellite groups were analyzed together for the treatment period and separately for the recovery period. There were no effects on mean body weight or body weight gain for males or females. Food consumption was significantly ($p \leq 0.01$) elevated in high-dose males (4-8%) and females (12-18%), during the treatment period. Food consumption tended to be slightly elevated in the mid-dose group and feed efficiency tended to be lower in the high-dose group, but these differences were not persistent during the treatment period. These differences completely reversed during recovery. **Hematology:** Although there were sporadic statistically significant differences between some dose and control groups for certain parameters, none were dose-related and all values were within the range of normal values. **Clinical chemistry:** There was a dose-related decrease of blood cholesterol levels for the mid- and high-dose males after 4 weeks of exposure. Mean values were reduced 18% and 25% in the mid and high dose groups respectively, compared to controls. Cholesterol levels returned to normal values during recovery. There were no other treatment-related differences of clinical chemistry parameters. All urinalysis parameters were unchanged. **Terminal organ and body weights:** Adrenal weight and adrenal/brain weight ratio were elevated for high-dose females at the end of the exposure period. A post-recovery increase in adrenal weight, adrenal/body weight and adrenal/brain weight ratios were observed for high-dose males, but female adrenal weights were in the normal range after recovery. Microscopic lesions of adrenals were observed in the reproductive phase of this study. Kidney/body weight ratio was slightly elevated (12.5%) for high-dose males at the end of exposure, but not after the recovery period. Absolute kidney weight and kidney/brain ratio were unaffected. The relative kidney weight differences were not accompanied by microscopic pathology findings in the reproductive phase of the study and are therefore not toxicologically significant. There were no differences of organ weights among males or females in the low- and mid-dose groups. **Macroscopic and microscopic pathology:** No dose-related macroscopic pathology or microscopic pathology in grossly abnormal tissues was observed at the 4-week exposure and recovery sacrifice times. Extensive microscopic examinations of central (including eyes) and peripheral nervous system tissues, showed no differences between high-dose and control males. All macroscopic tissue observations were considered incidental findings. |
| ***Conclusions*** | NOAEL = 300 mg/kg b.wt./day for males and females based on increased adrenal weights and increased food consumption without commensurate body weight gain |
| ***Data Quality*** | Reliable with restriction (Klimisch Code 2)<br>Comparable to guideline study with acceptable method restriction for subchronic hazard determination |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 5 Mar 2000 |

**Robust Summary 26-Repeated Dose-3**

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|---|---|
| *Test Substance* | |
| CAS # | CAS# 72275-86-6 |
| Chemical Name | Alkenes, C15-18 alpha, reaction products with sulfurized tetrapropenyl phenol, calcium salt, sulfurized |
| Remarks | Test material purity not provided. |
| *Method* | |
| Method/Guideline followed | OECD 410 |
| Test Type | A four week dermal toxicity study in rats |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1986 |
| Species | Rat |
| Strain | Sprague-Dawley CD, 6-9 weeks of age at study initiation |
| Route of administration | Dermal, 6 hour/day, to the clipped, unabraided, dorsal surface. |
| Duration of test | 21 days of treatment (Monday through Friday; 21 total doses administered/animal) |
| Doses/concentration levels | 0, 3, 10, and 30% in mineral oil |
| Dosing solution analysis | Prestudy homogeneity and stability and dosing solution analysis |
| Vehicle | Mineral Oil USP |
| Sex | Males and females |
| Dose volume | 1 ml/kg |
| Frequency of treatment | Once/day, 5 days/week |
| Control and treatment groups | 10 animals/sex/group. The control animals were administered the vehicle. |
| Post exposure recovery period | None |
| Dose range finding study | None |
| Statistical methods | Body weight, body weight gains, food consumption, hematology and clinical chemistry parameters, organ weights, organ/brain weight ratios and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test, Kruskal-Wallis and Mann-Whitney U-tests. |
| Remarks field for test conditions | **The test material was applied to the clipped, unabraided dorsal surface of the rats for 6 hours/day, 5 days/week for 21 days. On each dosing day the appropriate dosing suspension was applied to the shaved area. Animal fur was reclipped twice/week. A plastic collar was placed around each animal's neck to prevent ingestion of the test material. Following the 6-hour exposure the collars were removed and the application site was wiped with gauze moistened with mineral oil. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the suggested Guideline.)**<br><br>**Animals were examined for viability twice daily and for signs of** |

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| | **toxicity once daily. Detailed clinical observations were made weekly. Pupil response was evaluated pretest and on the first and last day of treatment. Dermal responses were evaluated (Draize) on the first and last day of treatment and weekly, on Fridays, during the study. Evaluations were performed approximately 30 minutes following the removal of the test material. Body weights were recorded twice weekly during the study. Food consumption was recorded weekly during the study. Hematology and clinical chemistry parameters were evaluated for 10 animals/sex/group at termination. Macroscopic examinations were performed on all animals. Select organs were weighed. The lungs, liver, kidneys and skin (treated and untreated) were examined microscopically from all animals. In addition the brain, adrenals, spleen, testes, ovaries, and gross lesions were examined microscopically from all animals in the control and high dose groups.** |
| *Results* | |
| Remarks | Dosing solution analysis confirmed that the dosing solutions were prepared accurately and were homogeneous and stable over their period of use.<br><br>No deaths occurred and no compound related signs of toxicity were observed during the study. The physical observations that were observed included intermittent ocular and nasal discharges in both sexes in all groups. Swollen eyes were also observed in both sexes in all groups concurrent with or secondary to eye discharge. Other findings included alopecia on the forepaws and scabs on the neck. All of these findings were attributed to the use of collars during treatment and were not considered compound related. Normal pupil responses were observed in all animals throughout the study.<br><br>Skin irritation was observed in most animals in all groups. Generally the incidence of irritation was higher in the mid and high dose groups than in the control and low dose groups. Slight to well defined erythema and slight edema were observed in the control and low dose groups. Slight to moderate erythema and edema were observed in the mid and high dose groups. The control and low dose group findings were attributed to a vehicle effect. Mid and high dose group findings were attributed to treatment with the test material. Flaky skin was observed in all groups and was not related to treatment with the test material.<br><br>Body weights, body weight gain and food consumption data were unremarkable during the study.<br><br>There were no treatment-related differences from control observed in the hematology data of the treated animals following the treatment period. Serum chemistry values were considered unremarkable in the treated animals at termination with the exception of mean triglyceride values that were significantly reduced, compared to control (38-42%) in the high dose males and females and the mean BUN/creatinine ratio in the high dose males that was significantly greater than control.<br><br>**There were no alterations in absolute or relative (organ/body weight, organ/brain weight) organ weights that were attributed to** |

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| | **treatment with the test material.**<br><br>Several gross pathological observations appeared sporadically and exhibited no dose related trends. They were not considered treatment related. Findings included scabbed skin in one low dose and two high dose males; dry, flaky skin in one low dose male and female; renal pelvis dilation in one male and two females from both the control and mid dose groups and tan areas on the liver in two males in the low dose group and in one male and two females in the high dose group. These findings were not considered treatment related.<br><br>Microscopic examination of treated skin sites showed trace to severe acanthosis and trace to moderate hyperkeratosis in the high dose males and females. These findings were considered treatment related. No compound related skin lesions were observed in the low or mid dose groups. Minimal acanthosis and hyperkeratosis in the males and acanthosis in the females were observed in the control, low and mid dose groups. These findings in the control, low and mid dose groups were attributed to the treatment procedure and not to the test substance. All other histopathological changes that were observed were spontaneous or naturally occurring lesions in rats of this strain. |
| *Conclusions* | Repeated dermal exposure over a four-week period resulted in skin irritation, histologic skin changes and decreases in serum triglyceride levels and increased BUN/creatinine ratio (males only) in the high dose group. Skin irritation was observed grossly in the mid dose level. The low dose group was considered the no observed effect level. The NOAEL for systemic toxicity is 100 mg/kg/day. |
| *Data Quality* | Reliable without restriction (Klimisch Code) |
| *References* | Unpublished confidential business information |
| *Other* | Updated: 3/6/2003 |
| | |

## 4.4 Genetic Toxicity

**Robust Summary 26-GeneTox-1**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 67762-55-4 |
| Chemical Name | Alkenes, C15-18 alpha, sulfurized |
| Remarks | This chemical is also referred to as C15-C18 alkene derivative in the HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Designed to be in compliance with microbial mutagenicity testing as set forth by OECD 1981, EPA 1982, FDA 1993 |
| Test Type | Reverse mutation assay |
| System of testing | Bacterial |
| GLP (Y/N) | Y, except analyses were not performed to verify the homogeneity, stability or accuracy of the test/control article preparation |
| Year (Study Performed) | 1996 |
| Species/Strain | Salmonella typhimurium – TA1535, TA1537, TA98, TA100 and TA102<br>Escherichia coli – WP2 uvrA |
| Metabolic activation | With and without |
| Concentrations | Prescreen, duplicate cultures: 50.0, 167, 500, 1670, and 5000 microgram/plate, plus control<br><br>Triplicate cultures: 50.0, 167, 500, 1670, 5000 and 10,000 micrograms/plate |
| Statistical methods | Statistical analyses are performed using the program developed by Snee and Irr (1981), with significance established at the 95% confidence limit. |
| Remarks field for test conditions | Test article was first evaluated in a prescreen using both liquid pre-incubation and plate incorporation treatment conditions. Duplicate cultures of strains TA1537, TA100, an dWP2 uvrA were treated with article at doses of 50.0, 167, 500, 1670, and 5000 micrograms/plate, as well as the solvent control, in the absence of S9. The test article was found to be incompletely soluable (droplets were observed) at all doses.<br><br>The article was next evaluated using both treatment conditions. Based upon the results of the prescreen, the article was evaluated in triplicate cultures in strains TA1535, TA1537, TA98, TA100, TA102, and WP2 uvrA in the presence and absence of S9 at doses of 50.0, 167, 500, 1670, 5000 and 10,000 micrograms/plate. Six doses of the article were evaluated in the event of unacceptable toxicity and/or insolubility at the highest dose levels evaluated in the mutation assay. The S9 mixture included 6% (v/v) Aroclor 1254-induced male Sprague-Dawley rat liver homogenate with the appropriate bugger and cofactors. The test article was again found to be incompletely soluble at all doses, under both treatment conditions. All positive and negative controls were within acceptable ranges. |
| ***Results*** | |
| Remarks | In the prescreen, results indicated that the article was not toxic.<br><br>In the following study, normal growth was observed in all tester strains at all doses evaluated with and without S9. Revertant frequencies for all |

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| | doses of article in all tester strains, with and without S9 under both treatment conditions approximated or were less than those observed in the concurrent negative control cultures. |
| ***Conclusions*** | The results were negative in this study, using liquid pre-incubation and plate incorporation treatments. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). No analyses to verify test article preparation. |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-29-99 |

**Robust Summary 26-GeneTox-2**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS # 91770-97-4 |
| Chemical Name | Alkyl (C12-C16) sulfide. |
| Remarks | This chemical is an analog to the C15-C18 alkene derivative (CAS # 67762-55-4; Alkenes, C15-18 alpha, sulfurized) in the HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | Method consistent with OECD 474 and EPA OPPTS 870.5395 |
| Test Type | Mouse micronucleus test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1996 |
| Species | Mice |
| Strain | B6C3F1 |
| Sex | Male |
| Route of administration | Intraperitoneal |
| Doses/concentrations | 0, 500, 1000, and 2000 mg/kg/day, plus negative control (vehicle = corn oil) and positive control (= cyclophosphamide) |
| Exposure Period | Three consecutive days |
| Statistical methods | Statistical analysis was not performed on the frequency of micronucleated PCEs since test article animals had lower average numbers of micronucleated PCEs compared to controls. |
| Remarks field for test conditions | There was a range-finding phase of the study, which consisted of four groups of two male mice/group. Dose levels were 0, 500, 1000, and 2000.<br><br>Groups of five mice each were dosed intraperitoneally with 0, 500, 1000, and 2000 mg/kg/day for three consecutive days and then sacrificed one day after the last dose. The positive control was administered as a single oral dose approximately 24 hours prior to sacrifice.<br><br>Bone marrow cells were analyzed for the number of polychromatic erythrocytes (PCEs) which contained at least one micronucleus. A minimum of 2000 PCEs were analyzed from each animal from the vehicle control and from mice dosed with the test article. A minimum of 1000 PCEs was analyzed from each animal dosed with the positive control. |
| ***Results*** | |
| Remarks | The test article, when dosed to mice at 500, 1000 and 2000 mg/kg/day for three consecutive days did not induce an increase in the number of micronuclei. There was an indication of slight bone marrow cytotoxicity at the highest dose in the micronucleus phase. The decrease was statistically different from the vehicle control. This decrease was due to the lower percentage of PCEs for two animals. The responses obtained from the negative and positive control articles confirmed the reliability that the test system was capable of detecting |

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| | compounds that induce micronuclei. |
| ***Conclusions*** | The test article did not cause an increase in micronuclei in developing erythrocytes in bone marrow from male B6F3C1 mice at the doses tested. There was a slight cytotoxic effect on developing erythrocytes at 2000 mg/kg/day, the maximum dose typically used in the mouse micronucleus phase. |
| ***Data Quality*** | Reliable without restrictions. |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-29-99 |
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**Robust Summary 26-Gentox-3**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 72275-86-6 |
| Chemical Name | Alkenes, C15-18 alpha, reaction products with sulfurized tetrapropenyl phenol, calcium salt, sulfurized |
| Remarks | Test material purity not provided. |
| ***Method*** | |
| Method/Guideline followed | OECD Guideline 471 |
| Test Type | Bacterial Reverse Mutation Assay |
| GLP (Y/N) | N |
| Year (Study Performed) | 1980 |
| Test System | **Salmonella typhimurium** |
| Strains Tested | *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 |
| Exposure Method | Plate incorporation |
| Test Substance Doses/concentration levels | 0.01, 0.1, 1.0, 10 mg/plate with and without activation (TA 98/TA 100)<br>0.1, 1.0, 10 mg/plate with and without activation (TA 1535/TA 1537) |
| Metabolic Activation | With and without (S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats) |
| Vehicle | Dimethylsulfoxide |
| Tester strain, activation status, Positive Controls and concentration level | TA98 +S9 2-aminofluorene 15 ug/plate<br>TA98 -S9 2-nitrofluorene 50 ug/plate<br>TA100 +S9 Benzo(a)pyrene 0.05-1.0 ug/plate<br>TA100 -S9 N-methyl-N-nitro-N-nitrosoguanidine 2.0 ug/plate<br>TA1535 +S9 2-aminoanthracene 20 ug/plate<br>TA1535 -S9 N-methyl-N-nitro-N-nitrosoguanidine 2.0 ug/plate<br>TA1537 +S9 2-aminofluorene 15 ug/plate<br>TA1537 -S9 9-aminoacridine 100 ug/plate |
| Vehicle Control | Dimethylsulfoxide |
| Statistical Analysis | Revertant colony count was determined for each dose point. |
| Dose Range finding Study | No |
| S9 Optimization Study | No |
| Remarks field for test conditions | This study was conducted prior to the development of OECD Guideline No. 471. This study deviates from the guideline in that E. coli WP2 urvA Tester Strain called for in the guideline was not include.<br><br>There were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with three or four concentrations of test substance, vehicle controls, and a positive control. Two plates/dose group/strain/treatment set was evaluated. 0.1 ml of test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, S9 mix (if needed) and 2.0 ml of top agar. This was overlaid onto the surface of minimal bottom agar in a petri dish. Plates were incubated for 48 to 72 hours at 37$^{o}$C. |

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| ***Results*** | The test substance was not genotoxic in this assay with or without metabolic activation. |
| Remarks | All data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation.<br><br>The positive control for each respective test strain confirmed the expected positive control response. |
| ***Conclusions*** | Under the conditions of this study, the test material was not mutagenic. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code). |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 3/07/2003 |
| | |

**Robust Summary 26-GeneTox-4**

| ***Test Substance*** | |
|---|---|
| CAS # | 122384-85-4 |
| Chemical name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide.<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenol sulfide and 40% highly refined lubricant base oil. |
| ***Method*** | |
| Method/guideline followed | Consistent with OECD 471 |
| Test type | Reverse mutation assay |
| System of testing | Bacteria |
| GLP (Y/N) | Y |
| Year (study performed) | 1996 |
| Species/strain or cell type/line | *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and *E. coli* WP2 uvrA |
| Metabolic activation | Species and cell type: Male Sprague-Dawley rat, liver S-9 fraction<br>Quantity: 50 µl/plate, 0.5 mL of 10% v/v S-9 mix per plate<br>Induced or not induced: Aroclor 1254 induced, 500 mg/kg i.p. |
| Concentrations tested | 0, 5, 10, 50, 100, 250, 500, 1000, 5000 and 1000 µg/plate, with and without S-9 |
| Statistical methods | Determination of mean ± S.D. of replicate plate counts |
| Remarks field for test conditions | Three replicate plates were evaluated for each treatment and results were confirmed in a second independent experiment. The vehicle for preparation of dosing solutions was 25% w/w Pluronic F127 (surfactant, CAS # 9003-11-6) in ethanol. Test material solubility limit in top agar administered in 0.1mL/plate was ≥5.0 µg /plate, however this did not interfere with scoring. Preliminary tests with TA 100 and WP2 uvrA showed no cytotoxicity with or without S-9 at doses of 6.67 - 5000 µg/plate. Acceptable range data was provided for untreated revertant rates. Vehicle was tested as negative control. Positive control treatments were as follows: |

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| | Strain / Activation / Positive Control / Amount/plate<br>TA 98 / + / Benzo(a)pyrene / 2.5 µg<br>TA 98 / - / 2-Nitrofluorene / 1.0 µg<br>TA 100 / + / 2-Aminoanthracene / 2.5 µg<br>TA 100 / - / Sodium azide / 2.0 µg<br>TA 1535 / + / 2-Aminoanthracene / 2.5 µg<br>TA 1535 / - / Sodium azide / 2.0 µg<br>TA 1537 / + / 2-Aminoanthracene / 2.5 µg<br>TA 1537 / - / ICR-191(CAS # 17070-45-0) / 2.0 µg<br>WP2uvrA / + / 2-Aminoanthracene / 25.0 µg<br>WP2uvrA / - / 4-Nitroquinoline-N-oxide / 1.0 µg<br>Valid test criteria stated in the report were: 1) phenotypic integrity of tester strains must be confirmed; 2) tester strain culture densities to be at least $0.5x10^9$ bacteria/mL; 3) positive controls to produce at least a three-fold increase in the number of revertants over the values for the respective negative controls; 4) a minimum of three non-toxic doses to be tested. Criteria for determination of no mutagenic activity: mean revertant colonies per plate < 2-fold higher than concurrent controls for strains TA 98, TA 100 and WP2 uvrA and < 3-fold higher for strains TA 1535, TA 1537.<br><br>All guideline recommendations were satisfied. |
| ***Results*** | |
| Result | Not mutagenic with or without metabolic activation |
| Cytotoxic concentration | With metabolic activation: > 10,000 µg/plate<br>Without metabolic activation > 10,000 µg/plate |
| Genotoxic effects | With metabolic activation: negative<br>Without metabolic activation: negative |
| Statistical results | Not applicable |
| Remarks | All test validity criteria were satisfied. There was no evidence of cytotoxicity based on condition of the background lawn or revertants/plate data at doses up to 10,000 µg/plate. The greatest mean number of revertants/plate over concurrent controls among all test doses, all strains and for both assays was 100% (TA 1537, next highest 81%) with S-9 and 100% (TA 1535 and 1537, next highest 47%) without S-9. There was no indication of a dose-related decrease or increase of mean revertants/plate. |
| ***Conclusions*** | Not mutagenic with or without metabolic activation |
| ***Data Quality*** | Reliable without restriction (Klimisch Code 1) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 5 Mar 2000 |

**Robust Summary 26-GeneTox-5**

| ***Test Substance*** | |
|---|---|
| CAS # | 122384-85-4 |
| Chemical name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide.<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 60% alkyl phenol sulfide and 40% highly refined lubricant base oil. |
| ***Method*** | |
| Method/guideline followed | Consistent with OECD 474 |
| Test type | Mammalian Erythrocyte Micronucleus Test |
| GLP (Y/N) | Y |
| Year (study performed) | 1996 |
| Species | Mouse |
| Strain | Crl:CD-1®(ICR) BR |
| Sex | Males and females |
| Route of administration | Intraperitoneal injection (i.p.) |
| Doses/concentration levels | 0, 1250, 2500 and 5000 mg/kg b.wt. |
| Exposure period | 24, 48 and 72 hours post-dose |
| Statistical methods | Results for each sex / harvest time were analyzed by ANOVA on either untransformed (when variance homogeneous) or rank transformed (when variance heterogeneous) micronucleus cell count data. If significance was observed with ANOVA, a Dunnett's t-test was used to determine which dose groups were different from the negative control. A Cochran-Armitage test for linear trend was used to evaluate dose-response. |
| Remarks field for test conditions | Doses were selected based on results of preliminary dose range finding studies. Five mice/sex/dose were tested at each harvest time. All mice were 8 weeks and 2 days old and initial body weights ranged from 23.7–35.1 grams for males and 21.2–28.2 grams for females. Dose solutions were prepared just prior to administration. Peanut oil was used for the test material vehicle and for treatment of negative controls. The dose volume was 10 mL/kg b.wt. for all groups. Cyclophosphamide at a dose of 60 mg/kg b.wt. served as the positive control. Animals were observed immediately after dosing and periodically throughout the study. At each harvest time, animals were euthanized by $CO_2$ inhalation; bone marrow was extracted from hind limb bones and prepared for microscopic evaluation. 1000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. The relative frequency of PCEs versus normochromatic erythrocytes (NCEs) was determined by scoring at least the first 1000 erythrocytes.<br><br>All guideline recommendations were exceeded, except for the following: Individual and group mean body weights were not reported and variation may have exceeded ± 20% of group means. 1000 instead of 2000 PCEs per animal were scored for micronuclei. |

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| ***Results*** | |
| Effect on mitotic index or PCE/NCE ratio by dose level and sex | There were no significant differences of group mean PCE/NCE ratios between treatment and control groups at any dose for all harvest times and for males and females. Likewise positive control PCE/NCE ratios were not affected. |
| Genotoxic effects | No increased proportion of micronucleated PCEs was observed in any test group. Both males and females of the positive control group had significantly elevated (>50-fold) proportion of micronucleated PCEs. |
| NOAEL (NOEL) (C)<br>LOAEL (LOEL) (C) | NOEL > 5000 mg/kg b.wt.<br>LOEL > 5000 mg/kg b.wt. |
| Statistical results | No statistically significant trends or differences from respective controls of treatment group means. |
| Remarks | There was no mortality and all animals appeared normal without sign of adverse effect until sacrifice. Mean percentage of micronucleated PCEs was within the range of laboratory historical controls for all treatment and vehicle control groups. |
| ***Conclusions*** | Not genotoxic in the mouse micronucleus assay |
| ***Data Quality*** | Reliable without restriction (Klimisch Code 1) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 5 Mar 2000 |

**Robust Summary 26-GeneTox-6**

| ***Test Substance*** | |
|---|---|
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | modified OPPTS 870.5395 |
| Test Type | Mammalian bone marrow erythrocyte micronucleus test; adjunct to 13 week dermal subchronic toxicity study |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1987 |
| Species | Rat |
| Strain | Sprague Dawley (Tac:N[SD]fBR) |
| Sex | Male and female |
| Route of administration | dermal to shaved skin of backs |
| Doses/concentrations | 500 and 2000 mg/kg/day undiluted test material; 500 mg/kg/day diluted 50%w/v in 100" mineral oil base stock<br><br>10 (5M,5F/group): 3 treatment groups,1 untreated controls<br>vehicle = mineral oil (100" solvent refined naphthenic base stock) density 0.88 g/ml |
| Exposure Period | 5 days/week for 13 weeks |
| Statistical methods | SAS ANOVA and ANOVA F test; Tukey's Studentized Range test and Scheffe's test; SAS General Linear Model, a studentized linear regression analysis to determine dose responsiveness. Statistical analyses compared test values to negative control data; a significant increase in micronuclei is an indicator of clastogenic activity by the test material |
| Remarks field for test conditions | Age at initiation: 7 weeks old following 2 weeks acclimation<br><br>Methyl propene derivative was applied to the clipped backs of groups of 20 Sprague Dawley rats (10M,10F) 5 days per week for 13 weeks at dose levels of 0, 500 or 2000 mg/kg/day undiluted or 500 mg/kg diluted (50% w/v) in 100" mineral oil base stock. Rats were fitted with Elizabeth collars to minimize ingestion of test material, which was left uncovered on the skin. At termination of the 13 week subchronic study, approximately 24 hours after the final dermal administration, bone marrow was harvested from femurs of the first 5 rats/sex/group necropsied. Three bone marrow slides were prepared for each animal. Slides were stained with acridine orange and scored under a fluorescence microscope. All slides were randomized by a computer-generated random numbers table so that the cytogeneticist was unaware of what dose group any individual slide was from. Immature red blood cells (polychromatic erythrocytes, PCE) and mature red blood cells (normochromatic erythrocytes, NCE) were evaluated for toxicity and the presence of micronuclei. The ratio of PCE to NCE per the first 1000 erythrocytes counted was calculated to determine cytotoxicity if any. At least 1000 PCE and 1000 NCE were scored for |

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| | the presence of micronuclei. |
| ***Results*** | |
| Remarks | Methyl propene derivative undiluted (500 or 2000 mg/kg/day) and methyl propene derivative (500 mg/kg/day) 50% w/v in 100" mineral oil base stock were not cytototoxic to red blood cell formation. These test materials did not induce any statistically significant increase in the formation of micronucleated PCEs or NCEs in bone marrow red blood cells of male or female rats exposed dermally for 13 weeks. |
| ***Conclusions*** | Methyl propene derivative does not cause chromosome damage in rats following regular and prolonged dermal exposure in this test system. NOEL= 2000 mg/kg/day |
| ***Data Quality*** | Reliable with restrictions. Study does not include a positive control. Samples were collected only once approximately 24 hours after the final dose. Information on test material composition, purity and stability are not part of this report but are referred to the 13 week subchronic study report. |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-29-99 |
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**Robust Summary 26-GeneTox-7**

| ***Test Substance*** | |
|---|---|
| CAS # | 68511-50-2 |
| Chemical Name | 1-propene, 2-methyl-, sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for Alkyl Sulfide Category.<br>For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/Guideline followed | OECD 474. |
| Test Type | Mammalian bone marrow erythrocyte micronucleus test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1989 |
| Species | Mouse |
| Strain | B6C3F1 |
| Sex | Male and female |
| Route of administration | Intraperitoneal |
| Doses/concentrations | Single injection of 3.5 g/kg test material, 50 mg/kg cyclophosphamide in saline, or methylcellulose vehicle alone<br>(vehicle = hydroxypropyl methylcellulose (Methocel K4M Premium - Dow Chemical))<br><br>15 animals (5M, 5F/dose/sample interval); Positive control (5M,5F) |
| Exposure Period | Single dose |
| Statistical methods | Normal test for equality of proportion (one-tailed). Because of multiplicity of comparisons , a Dunnett adjustment was made. |
| Remarks field for test conditions | Young male and female mice were treated with a single intraperitoneal injection of 3.5 g/kg test material, 50 mg/kg cyclophosphamide in saline, or methylcellulose vehicle alone. Dose had been determined in a preliminary toxicity test to identified MTD for this study. Animals were sacrificed and femurs removed at 24, 48 or 72 hours post dosing (5M, 5F per interval) for test material and negative control, and at 24 hours postdosing only for cyclophosphamide. Bone marrow smears were prepared and immature red blood cells (polychromatic erythrocytes, PCEs) and mature red blood cells (normochromatic erythrocytes, NCEs) were evaluated for toxicity and the presence of micronuclei. Slides were stained with acridine orange and scored under a fluorescence microscope. Slides from all dose groups were sorted by a computerized random number system and the cytogeneticist was unaware of what dose group any individual slide was from. The ratio of PCE or NCE per the first 1000 erythrocytes counted was calculated to determine cytotoxicity if any. |
| ***Results*** | |
| Remarks | In the preliminary toxicity test (2M, 2F/group) all mice died at 5.0 g/kg and all survived at 3.5 g/kg with no cytotoxicity in bone marrow cells 24 hours after injection. Data from the full study demonstrate that the frequency of mironucleated PCEs in femoral bone marrow for males and females treated with the test material was not significantly elevated ($p<0.05$) when compared to negative controls for groups |

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| | sampled at 24, 48 or 72 hours postinjection. Results from both sexes combined demonstrate the same results. Cyclophosphamide, the positive control material did induce statistically significant increases in micronucleated PCEs in all animals demonstrating that a valid study was performed. |
| ***Conclusions*** | Methyl propene derivative administered IP at 3.5 g/kg body weight did not induce the formation of micronuclei in PCEs in male or female mice at any time interval and is not considered clastogenic in this test system. |
| ***Data Quality*** | Reliable without restrictions. Guideline study. |
| ***References*** | This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information). |
| ***Other*** | Updated: 12-29-99 |
| | |

**Robust Summary 26-GeneTox-8**

| ***Test Substance*** | |
|---|---|
| CAS# | 68511-50-2 |
| Chemical name | 1-propene, 2-methyl- sulfurized |
| Remarks | This substance is also referred to as methyl propene derivative in HERTG's Test Plan for the Alkyl Sulfide Category. For more information on the chemical, see Section 2.0 "Chemical Description of Alkyl Sulfide Category" in HERTG's Test Plan for Alkyl Sulfide Category. |
| ***Method*** | |
| Method/guideline followed | Consistent with guidelines outlined in OECD 471 |
| Test Type | Reverse Mutation Assay |
| System of testing | Bacterial |
| GLP (Y/N) | No |
| Year (study performed) | 1978 |
| Species/Strain | *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98 |
| Metabolic activation | Test conducted with and without metabolic activation<br>Adult male Sprague-Dawley rat liver S-9 fraction, induced with Aroclor 1254<br>100 ul/plate |
| Concentrations | 0, 0.01, 0.05,0.1, 0.5, 1.0 ul of test agent per plate with and without metabolic activation |
| Statistical Methods | Determination of mean ±S.D. of replicate plate counts |
| Remarks Field for Test Conditions | The vehicle was DMSO;<br>All stock and working solutions were stored at 4°C in glass screw-capped bottles;<br>All sterility controls were negative for bacterial growth;<br>Vehicle was tested as negative control;<br>Positive controls (9-aminoacridine and 2-nitrofluorene without activation and 9-aminoacridine, 2-nitrofluorene, aflatoxin, and 6-aminochrysene with activation) were at least 3 times the number of colonies as the control. |
| ***Results*** | |
| Remarks | For all strains and dose levels with and without metabolic activation, the criteria for a positive mutagens (at least 3 times the number of colonies as the controls for spontaneous reversion) was not met. |

## 4.5 Reproductive and Developmental Toxicity

### Robust Summary 26-Repro/Devel-1

| ***Test Substance*** | |
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| CAS # | CAS# 67124-09-8 |
| Chemical Name | 2-Propanol, 1-(tert-dodecylthio)- |
| Remarks | Purity: 100 % |
| ***Method*** | |
| Method/Guideline followed | OECD 415 |
| Test Type | Oral (Gavage) One-Generation Reproductive Toxicity Study |
| GLP (Y/N) | Y |
| Year (Study Performed) | 2001 |
| Species | Rat |
| Strain | Sprague-Dawley Crl: CD®(SD) IGS BR rats, 71 days of age at initiation of treatment |
| Route of administration | Orally by gastric intubation |
| Duration of test | $F_0$ males- 70 days premating; mating period through completion of parturition.<br>$F_0$ females- 14 days premating; mating; 25 days of gestation and 20 days of lactation.<br>$F_1$ pups- gestation through day 20 of lactation. |
| Doses/concentration levels | 0, 50, 167 and 500 mg/kg/day |
| Vehicle control | Corn Oil |
| Dose volume | 2.5 mL/kg |
| Sex | Males and females |
| Frequency of treatment | Once/day, 7 days/week |
| Analytical confirmation of concentration. | Homogeneity, stability and weekly dose concentration confirmation. |
| Control and treatment groups | 28 $F_0$ rats/sex/group in the control, low, mid and high dose groups. |
| Mating | 1 male mated to 1 female from the same group until evidence of mating (presence of copulatory plug or sperm) was observed. If evidence of mating was not observed mating was discontinued after three weeks. |
| Post exposure observation period | None |
| Statistical methods | Pup ratios, pup survival indices, mean number stillborn and dead pups and parental fertility indices were evaluated using the Chi-square test. $F_0$ body weights and gains, gestation and lactation body weights and gains, parenteral food consumption, mean litter weights, length of gestation, live litter size and organ weights were evaluated using ANOVA (two-tailed) with Dunnett's test. Histopathological findings were evaluated using the Kolmogorov-Smirnov (one-tailed) test. Post implantation loss was analyzed using the Mann-Whitney U Test. |
| Dose range finding study | None |

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| Remarks field for test conditions | *$F_0$ Generation:*<br>All $F_0$ males were dosed for 70 days prior to mating and through the completion of parturition. All $F_0$ females were dosed for 14 days prior to mating and through day 20 of lactation. All $F_0$ animals were examined twice daily for appearance and behavior. Detailed clinical observations were performed weekly and cage side observations were performed daily approximately thirty to ninety minutes post dosing. Body weights were recorded weekly for both sexes prior to mating; maternal body weights were recorded on gestation days 0, 7, 14 and 21 as well as on lactation days 1, 4, 7, 14 and 21. Food consumption was recorded on the same days as body weights except during mating the period and during lactation. Animals were paired 1:1 for mating. Positive evidence of mating was confirmed by the presence of sperm or a vaginal copulatory plug (day 0 of gestation). If evidence of mating was not present after three weeks, mating was discontinued. All of the surviving $F_0$ females were allowed to deliver and rear their pups to lactation day 21. The offspring were potentially exposed to the test substance *in utero* and through nursing during lactation days 1-21 until euthanization on post-natal day 21. Hematology evaluations were performed on 10 males and 10 females /group prior to their scheduled termination. The surviving $F_0$ dams were necropsied on lactation day 21, following a minimum of 60 days of dosing. The surviving $F_0$ males were necropsied at the conclusion of parturition following a minimum of 96 days of dosing. The $F_0$ females with total litter loss were necropsied within 24 hours. $F_0$ females that failed to deliver were necropsied on post-mating day 25 (with evidence of mating) or 25 days following the termination of the mating period (with no evidence of mating).<br><br>**Organ weights were determined and microscopic examinations were conducted for all surviving control and high dose $F_0$ animals. Tissues examined microscopically included the liver, kidney, brain, right epididymides, cervix, coagulation gland, ovaries, pituitary, prostrate, seminal vesicles, testes, uterus, vagina and gross lesions.**<br>**$F_0$ animals from all groups found dead or sacrificed early were subjected to a gross necropsy and the microscopic evaluation of all tissues.**<br><br>**Sperm was collected from all surviving $F_0$ males and evaluated for sperm count, concentration, motility and morphology assessment.**<br><br>***$F_1$ Generation:***<br>**On lactation day 4 each litter was randomly culled to a maximum of eight pups, 4/sex/litter, when possible. Detailed pup examinations were performed on lactation days 0, 4, 7, 14 and 21. Pup sex was determined on lactation day 0 and verified on lactation days 4, 7, 14 and 21. Individual pup weights were determined on lactation days 1, 4, 7 14 and 21. Pups that were stillborn, cannibalized or found dead were subjected to a gross necropsy with emphasis on developmental morphology. Pups culled on day 4 were subjected to an abbreviated gross necropsy with emphasis on the reproductive system. All surviving pups were euthanized on lactation day 21 and examined** |

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| | **macroscopically. All internal gross lesions were preserved for possible future microscopic examination.** |
| ***Results*** | Results of the homogeneity analysis indicate that the test article was homogeneous in the vehicle and stable for more then one week when stored under ambient conditions. Concentration analysis confirmed that the test article was at the appropriate concentration in the dosing solutions.<br><br>*$F_0$ Generation:*<br>$F_0$ males exhibited a significant increase in post dosing salivation in the mid and high dose groups and lower mean body weights (5-7% compared to controls) in the high dose group. The remaining $F_0$ male parameters were unremarkable including: mean food consumption, mating and fertility indicies, hematology data, absolute and relative organ weights, sperm evaluation parameters and macro and microscopic pathology.<br><br>$F_0$ females in the low, mid and high dose groups exhibited a low incidence of reddish vaginal discharge, mammary gland swelling and dark material around the eyes and nose. The mid and high dose females exhibited a low incidence of salivation prior to dosing, an increased incidence of urine stain and a dose related increase in post dosing salivation. The high dose females also exhibited a low incidence of ocular discharge. Two females in the low dose group delivered all stillborn pups. The number of females with live born pups was 25, 24, 27 and 26 in the control, low, mid and high dose groups respectively. There were no toxicologically meaningful differences between the control low, mid and high dose groups with respect to $F_0$ female mean body weights, body weight change, food consumption, mating and fertility indicies, precoital intervals, gestation length or mean hematology values. Macroscopic findings observed in one high dose female sacrificed on post breeding day 25 included dark red/brown fluid in the uterus and vagina, and one small placenta and two nonviable pups in the vagina. No other remarkable findings were noted in the $F_0$ females at necropsy and no meaningful microscopic lesions were observed in any of the treated $F_0$ females.<br><br>*$F_1$ Generation:*<br>The total and mean number of $F_1$ pups delivered was comparable between the control and treated groups. However the number of $F_1$ liveborn pups in the low dose group was statistically lower than control. This was attributed to the higher number of still born pups, primarily from two females in this group that experienced total litter losses. The number of live pups/litter was comparable between the control and treated groups throughout lactation. Sex ratios were comparable to control in all treated groups on lactation days 0 and 21. Mean pup weights were statistically lower than control in the mid dose group on lactation |

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| | day 14 and in the high dose group on lactation days 14 and 21, however, the mean pup weights of these groups remained within the range of the test facilities historical control data. With the exception of a slight increase in the incidence of pups that were cool to the touch, no remarkable pup observations were noted during lactation.. No significant necropsy findings were noted for stillborn pups, pups found dead, pups culled on day 4 or pups sacrificed on lactation day 21. |
| ***Conclusions*** | Based on the results of this study the Study Director concluded that the 50 mg/kg/day dose level was the no observed adverse effect level (NOAEL) for parental $F_0$ toxicity. There were no indications of impaired fertility or other reproductive effects in the parental males or females at doses up to 500 mg/kg/day. A dose level of 50 mg/kg/day was considered the (NOAEL) for developmental effects, as a result of decreased pup weights in the mid and high dose groups during the latter half of gestation. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 1/20/2003 |
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**Robust Summary 26-Repro/Devel-2**

| ***Test Substance*** | |
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| CAS # | 122384-85-4 |
| Chemical name | Phenol, dodecyl-, sulfurized, calcium salts. |
| Remarks | This substance is referred to as dodecyl derivative in the HERTG's SIAR for Alkyl Phen(ol)ate Sulfide Category.<br>For more information on the chemical, see Section 1.0 "Chemical Description of Alkyl Phen(ol)ate Sulfide Category" in HERTG's SIAR for Alkyl Phen(ol)ate Sulfide.<br><br>Testing was performed on a commercial sample of this material. Typical purity of this material as distributed in commerce is 75% alkyl phenol sulfide and 25% highly refined lubricant base oil. |
| ***Method*** | |
| Method/guideline followed | OECD 422 (draft) |
| Test type | Oral reproduction/developmental toxicity screening study in rats |
| GLP (Y/N) | Y |
| Year (study performed) | 1996 - 1997 |
| Species | Rat |
| Strain | Sprague-Dawley, Crl:CD® BR, $F_0$ age 52 days at initiation of treatment and 82 days at mating |
| Route of administration | Oral gavage |
| Doses/concentration levels | 0, 50, 300 and 1000 mg/kg b.wt./day administered in a dose volume of 5 mL/kg b.wt./day |
| Sex | Males and females |
| Control group and treatment | 12 rats/sex received corn oil vehicle only |
| Frequency of treatment | Daily |
| Duration of test | 10 weeks |
| Premating exposure period for males (P and $F_1$) as appropriate | $F_0$ (P) - 31 days |
| Premating exposure period for females (P and $F_1$) as appropriate | $F_0$ (P) - 31 days |
| Statistical methods | Body weight, food consumption, feed efficiency, organ weight, gestation interval and litter data were analyzed with Bartlett's test to determine homogeneity of variances. If group variances were equal, data were analyzed by ANOVA with a Dunnett's test if significance was observed with ANOVA. If group variances were unequal, data were analyzed by the non-parametric Kruskal-Wallis test with a Dunn's rank sum test if significance was observed with Kruskal-Wallis. A test for dose-response trend was also conducted using regression analysis for parametric data and Jonckeere's test for monotonic trend for non-parametric data. Mating and male fertility indices and pregnancy rates were analyzed with Fisher's Exact test with the Bonferonni correction. |
| Remarks field for test conditions | Corn oil dosing solutions were prepared weekly, and their test material concentration, homogeneity and stability verified by chemical analysis. There were 12 rats/sex/group for the $F_0$ generation. Male and female parental animals were dosed daily during the pre-mating (31 days), mating (15 days), gestation (20 to 22 days) and lactation (4 |

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| | days) periods until necropsy. $F_0$ animals were paired within their groups on a 1:1 basis for mating. Females were examined daily during mating for presence of a copulatory plug or sperm in the vagina. When evidence of mating was not detected within 10 days, the female was placed for up to 5 days with another male from the same group that had previously mated. Animals were observed twice daily (morning and afternoon) and one hour after dosing during the administration period for mortality or moribundity. Detailed clinical observations were recorded weekly. Individual body weights were recorded twice pre-study, weekly during exposure and at termination. Food consumption was recorded twice pre-study and weekly during exposure, except during mating and for females during the gestation and lactation period when shorter intervals were used. At completion of parturition, litters were examined for viability, sex and gross malformations. Litters were observed twice daily (morning and afternoon) until lactation day 4. Pups were sexed and weighed on lactation days 0 and 4. All parental animals and pups were euthanized with carbon dioxide inhalation. Females and their litters were terminated on lactation day 4 and parental males were terminated after 70 days of treatment. Necropsy of pups included macroscopic internal and external examinations and abnormal tissues were preserved. All pup skeletons were processed for future skeletal examinations. Complete necropsy examinations were performed on parental animals. Sperm assessments (motility, caudal sperm count and morphology) were conducted on all parental males. Eleven major organs were weighed and 27 organs / tissues were preserved for all parental animals. Microscopic examination of the following tissues were made for all parental animals in the control and high-dose groups: tissues with macroscopic findings, adrenal glands, brain (medulla/pons, cerebellar and cerebral cortex), epididymides, heart, kidneys, liver, ovaries and oviduct, pituitary gland, seminal vesicles, spleen, and testes.<br><br>All guideline recommendations were exceeded, except for the following: Heart weights were not measured. Microscopic observations were not made for the following tissues: stomach, small and large intestines, thymus, trachea, lymph nodes and bone marrow. |
| ***Results*** | |
| NOAEL (NOEL) and LOAEL (LOEL) for P, $F_1$ and $F_2$ as appropriate | Reproductive toxicity NOAEL = 1000 mg/kg b.wt./day for males and females, highest dose tested<br>Developmental toxicity NOAEL = 1000 mg/kg b.wt./day, highest dose tested.<br>Subchronic toxicity NOAEL = 300 mg/kg b.wt./day for males and females<br>Subchronic toxicity LOAEL = 1000 mg/kg b.wt./day for males and females based on: 1)decreased body weight gain in males, 2) microscopic pathology and increased weight of adrenal glands in females, 3) increased food consumption without commensurate body weight gain in males and females |
| Actual dose received by dose level by sex if available | 0, 50, 300 and 1000 mg/kg b.wt./day |
| Parental data and $F_1$ (toxic | Decreased body weight gain and increased food consumption without |

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| response/effects with NOAEL value) | commensurate body weight gain in males after 8-10 weeks treatment at 1000 mg/kg b.wt./day. Fine vacuolar change indicative of lipidosis in the zona fasiculata of the adrenal cortex, increase adrenal weight and increased food consumption without commensurate body weight gain in females at 1000 mg/kg b.wt./day. NOAEL for all effects in males and females was 300 mg/kg b.wt./day. |
| Offspring toxicity $F_1$ and $F_2$ as appropriate (toxic response/effects with NOAEL value) | No treatment-related effects in offspring. Developmental toxicity NOAEL in offspring was 1000 mg/kg b.wt./day, highest dose tested. |
| Statistical results | Mean body weight gain for high-dose males was significantly lower than controls for weeks 8 ($p \leq 0.05$) and 9 ($p \leq 0.01$). Food consumption for high-dose males was significantly higher than controls during weeks 8-10 ($p \leq 0.01$). Feed efficiency was significantly lower for high-dose males during weeks 8 and 9 ($p \leq 0.01$). Food consumption was significantly higher for the high-dose females during pre-mating weeks 2 ($p \leq 0.01$) and 3 ($p \leq 0.05$) and gestation days 7-14 ($p \leq 0.01$). Mean adrenal weights in high-dose females were significantly higher ($p \leq 0.05$) than controls. There were no other statistically significant differences of toxicological significance. |
| Remarks | The homogeneity and stability of test material in corn oil dosing solutions were confirmed by chemical analysis of calcium content. Measured concentrations of test material in dosing solutions collected during the study were within 15% of nominal concentrations. **Survival:** All animals survived to termination. **Clinical observations:** No treatment-related clinical signs were observed. **Body weights and food consumption:** Mean body weights for high-dose males were lower (plateau 7-9%) than controls during the 10-week treatment period, but the differences were not statistically significant. Mean body weight gain for high-dose males was significantly lower than controls for weeks 8 ($p \leq 0.05$) and 9 ($p \leq 0.01$). Food consumption for high-dose males was significantly higher (12-27%) than controls during weeks 8-10 ($p \leq 0.01$). These changes made feed efficiency significantly lower for high-dose males during weeks 8 and 9($p \leq 0.01$). Female body weights were not affected during the 4-week pre-mating exposure period, but ~9% higher food consumption was recorded for the high dose group, which was statistically significant on weeks 2 and 3. Also during gestation and lactation, maternal body weights were not affected, while food consumption was elevated 8-17% in the high-dose group during gestation ($p \leq 0.01$, gestation days 7-14). Differences were not seen in maternal food consumption during lactation. **Reproductive parameters:** Male fertility, male and female mating indices, and female pregnancy rates were not affected by treatment. There were no treatment-related differences among group mean percent motile sperm, caudal epididymal sperm counts and percent abnormal sperm. The mean number of days until mating was comparable among all groups. The gestation index was 100% in all groups and length of gestation was not affected by treatment. There was no indication of prolonged gestations or protracted deliveries in any treatment group. **Litter data:** The number of corpora lutea and implantation sites were not affected by treatment. The mean number of pups/litter, the live birth index, sex ratio and viability index were also unchanged. There were no significant differences of male and female pup weights at days 0 and 4 of lactation. No pups were malformed. **Terminal organ and body** |

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| | **weights:** Kidney, liver and testes organ/body weight ratios were significantly higher in high-dose males than controls, while absolute and organ/brain ratios were not different. There were no microscopic pathology findings for these tissues. Therefore the elevated organ/body weight ratios in high-dose males are not toxicologically significant. Mean adrenal weights in high-dose females were 17% higher than controls. Relative adrenal weights were also elevated. The difference in absolute weights was statistically significant ($p \leq 0.05$), but not for relative adrenal weights. No other parental organ weights were affected by treatment. **Macroscopic and microscopic pathology:** No treatment-related macroscopic pathology was observed in parental animals or pups sacrificed at termination or found dead. The only treatment-related microscopic finding was seen in the adrenal glands of parental females. Fine vacuolar change indicative of lipidosis was observed in the zona fasiculata of the adrenal cortex for most animals in all groups. Severity was minimal to slight in most controls, low and mid-dose groups. In the high-dose group severity was mostly moderate. |
| ***Conclusions*** | Reproductive toxicity NOAEL= 1000 mg/kg b.wt./day for males and females, highest dose tested.<br>Developmental toxicity NOAEL= 1000 mg/kg b.wt./day, highest dose tested.<br>Subchronic toxicity NOAEL = 300 mg/kg b.wt./day for males and females based on decreased body weight gain in males, microscopic pathology and increased weight of adrenal glands in females, and increased food consumption without commensurate body weight gain in males and females |
| ***Data Quality*** | Reliable with restriction (Klimisch Code 2)<br>Comparable to guideline study with acceptable method restrictions only affecting subchronic hazard determination |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 5 Mar 2000 |